The new antiepileptic drugs - the search for synergy

A thesis by

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Dedication

To Jack, Matthew and Roisin for letting me use the computer at home occasionally. Oh, and thanks for the noise - it gave me an excuse to take a break when I got fed up.

Special thanks to my parents who have always encouraged me in everything I do.

Lastly, and mostly, to Veronica: I suspect that finishing this is more of a relief for you than for me. Without your help this would be, at best, a pamphlet. Thanks for ever. Now it's your turn!

Declaration

The work for this thesis was carried out during my tenure as a research fellow in the Epilepsy Research Unit, University Department of Medicine and Therapeutics, Western Infirmary, Glasgow. All studies reported have been published, submitted for publication or being prepared for publication. A list of these papers is included as is a list of presentations to learned societies. Reprints, where available, are enclosed.

The work was greatly facilitated by the efforts of many friends and colleagues who are formally acknowledged. The remainder of the work was carried out by myself, as was most of the statistical analysis, unless otherwise indicated. The writing of this thesis was entirely my own work.

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Finally, thanks to Tom Muir who patiently resolved my computing hiccups which were invariably a case of the uninformed wrestling with the unintelligible. From now on, I promise to read the manuals!

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INTRODUCTION

Summary

Many epileptologists believe that the new antiepileptic drugs as a group constitute a major advance in the treatment of epilepsy. Even their fiercest proponent, however, could not convincingly argue that they are the ideal treatment for all patients with epilepsy. It is widely accepted that if the 'magic bullet' for epilepsy exists, then we have still to find it. Given that the development of new antiepileptic compounds is an expensive, time-consuming gamble, then it may be more beneficial to expend our energies on a quest for better ways of using those compounds already available. Combination therapy is commonplace in other conditions such as hypertension, Parkinsons disease, cardiac failure, or infections such as tuberculosis, and there should be no reason why specific treatments should not be combined with particular efficacy in the treatment of epilepsy.

Polypharmacy with antiepileptic drugs (AEDs) has fallen out of favour in recent times. For some years, the prevailing opinion has been that AED combinations merely maximise the incidence of drug-related adverse events while conferring little benefit in terms of seizure control. While this may have been true of the established drugs, phenytoin, carbamazepine, and valproate we should be open to the possibility that the newer AEDs are more suited to use as polypharmacy.

We know that the newer AEDs have different mechanisms of action, have fewer pharmacokinetic interactions, and cause less sedation than their older counterparts, while pre-clinical trials would also suggest that they are more

specific in their actions. These qualities may suggest that there will be a reduction in the frequency and / or severity of pharmacodynamic interactions when they are used simultaneously.

Aside from chance observation, how else can we begin to plan our treatment combinations? The results of basic in-vitro research may suggest certain possibilities, but these will only be relevant in our clinics if we are aware of the key issues involving each new AED. Most importantly:

- *What is/are the relevant mode(s) of action of each drug?*

To anticipate pharmacodynamic interactions and therapeutic synergy, we need to have a comprehensive view of the actions of each individual drug. For example, vigabatrin is known to inhibit GABA-transaminase, but what changes does it exert on the metabolism of glutamate? Does it have other important effects on GABA? Remacemide, another AED in development was known to be a non-competitive n-methyl-d-aspartate (NMDA) receptor antagonist, but recent evidence has proven its effect on sodium channel conductance. What effect will it have on the GABAergic system?

Even once we have a fuller picture of each drug's neurochemical effects (and we cannot even do this convincingly for those drugs that are licensed in the UK!) then how do we proceed in planning the treatment of refractory epilepsy? Should we combine drugs which target the same system (e.g. two GABAergic drugs)? Or should we aim to manipulate two different systems (e.g. one drug acting on the GABAergic system, and one on the excitatory system)?

- *Are there any pharmacodynamic or pharmacokinetic interactions between*

the two drugs? Are these effects beneficial or deleterious?

The answer to this can only be gleaned from proper testing during preliminary clinical trials. Each new AED firstly has to undergo clinical trials as add-on therapy; pharmacokinetic interactions are usually picked up at this relatively early stage of investigation. During each trial, it may also be rational to carry out meta-analyses to investigate which co-therapies are particularly well or poorly tolerated. Despite the relative ease with which this might be accomplished in this age of computerisation, no such analyses have been carried out (or at least not been published!) for any of the emergent treatments, and possible reasons for this will be discussed later.

Once both questions are satisfactorily answered, and we suspect that a particular combination of drugs may have some particular merit, then further testing against appropriate controls will be required. The difficulties involved in this will be addressed.

The experiments described in this thesis investigate different aspects of some of the newer AEDs. Animal experiments are used to investigate the neurochemical actions of remacemide, gabapentin, tiagabine, and vigabatrin at varying doses with particular emphasis on the GABA shunt. Following the clinical observation of good additive effect with combined tiagabine and vigabatrin, the same parameters were used to look at this particular combination. Culture of rat astrocytes and neurones are used to delineate the dose-related effects of vigabatrin on GABA uptake, a phenomenon previously described in our laboratory. The specific combination of vigabatrin and

tiagabine is also used to search for additive or synergistic effects on this system.

One set of double-blind clinical studies attempts to investigate interactions between remacemide and the established AEDs, while another investigates the cognitive effects of add-on gabapentin.

The issues faced by clinicians in the formation of a rational plan for AED polypharmacy are discussed, and the scope for further investigation is explored.

New antiepileptic drugs - the Search For Synergy

Introduction

Epilepsy is a common neurological condition, with a lifetime prevalence of between two (Goodridge and Shorvon 1983a, Goodridge and Shorvon 1983b), and five (Shorvon 1990) percent of the general population, and a point prevalence of between 4 and 80 per 1000 (Brodie and Dichter 1996). There are an estimated fifty million cases world-wide, and even today the disease remains both disabling and stigmatising (Shorvon 1990). Even in the best centres, full seizure control is not guaranteed; most recent studies still suggest that around 30% of all patients will be inadequately controlled when using maximum tolerated doses of currently available AEDs (Beghi et al 1986, Schmidt 1984, Brodie and Dichter 1996).

Epilepsy has been recognised as a clinical entity for thousands of years, the name deriving from the ancient Greek word *epilambanein* meaning 'to seize' or 'to attack', and should correctly be regarded not so much as a disease, but as a symptom with myriad causes. The dysfunction which leads to clinical seizure activity may arise as a result of some primary, local event such as traumatic damage, inflammatory change or ischaemic events, or there may be an innate predisposition to seizure activity by virtue of a genetically-driven change in neuronal membrane activity. The relative contributions of nature and nurture to the ictal tendency will vary from patient to patient. The excessive discharge which is the root cause of the ictal phenomenon may remain localised, producing partial seizures, or may spread, producing generalised seizures.

The earliest writings on epilepsy date back to the Babylonians and the Chinese in 700 BC (Kinnier Wilson and Reynolds 1990, Lai and Lai 1991). Although early civilisations such as the Babylonians correctly associated epilepsy with physical illness such as head trauma (Gross 1992), the disorder was more usually ascribed to forces as irrelevant as evil spirits or lunar phases. Cautery, skull trephination, dietary manipulation, phlebotomy and good old-fashioned exorcism were therefore regarded as reasonable remedies until well past the middle ages.

Despite the ready availability of scientific explanations for it's basis, western societies continue to harbour a widespread ignorance and superstition about epilepsy which is all too familiar to sufferers and their families. At least this is an improvement. In ancient times, stigmatisation was endorsed by physicians: Pliny's advice included spitting on afflicted patients to "throw off the contagion" (Tempkin 1971), a practice that would hopefully not be condoned today!

The first physician to attempt a rational as opposed to supernatural explanation for epilepsy was Hippocrates in around 400 BC: he considered that there was an "excess of phlegm (that) overspilled into the bloodstream" (Tempkin 1971a). By the end of the second century, seizures were being classified according to their clinical manifestation, and around that time, Galen recorded the symptoms associated with the onset of seizure activity, calling them 'auras' a name by which they still go today. He had tried to fuse his observations on the nature of auras with the prevailing theories of interactions between body humours in an attempt to provide answers to the

clinical questions that confronted him (Tempkin 1971b).

Probably fuelled by it's association with divine intervention in the New Testament (Gospels of Mark and Luke), the direction of epilepsy management in the 'civilised' world remained with the clerics rather than the medics right up until the Enlightenment. The Middle Ages did not constitute one of medicine's golden eras, and little progress was made in the development of rational thinking in terms of epilepsy.

By 1780 Home had recognised the failings of modern medicine to counteract the superstitions of the day, and he was part of a general attempt to look closer to earth for an explanation of epilepsy aetiology, and a rational basis for it's cure. Leuret and Moreau (Gross 1992) disproved the idea that lunar phases were a significant factor in epilepsy aetiology, and useful observations were made by physicians such as Bright and Todd in linking focal seizures with focal central nervous system pathology (Gross 1992). Hughlings Jackson was able to relate these observations with the contemporary experiments that showed that electrical stimulation of discrete areas of cortex was related to specific motor effects. In doing this he was able to state that:

> *"A convulsion is but a symptom, and implies only that there is an excessive and disorderly discharge of nerve tissue on muscles."* (Jackson 1873)

The cause of seizures was still not well understood, and most authorities believed that hysteria and onanism were important factors in the development

of an ictal tendency. Work by Fritsch and Hitzig (De Villiers 1993) was pivotal in establishing the link between epilepsy and localised cerebral pathology. The discovery of weak electrical currents from the brains of rabbits and monkeys preceded the development of electroencephalography in 1929, and with the association of electrical activity and neuronal dysfunction confirmed, not only was the organic nature of epilepsy asserted, but the development of animal models would allow for more efficient preclinical testing of potential AEDs.

Despite the proven organic causes of this common disorder, the social stigma which still surrounds epilepsy is among the many factors which leads to the perception of disablement of some epilepsy patients. Surveys have shown that even among contemporary physicians, there is an erroneous belief that (Beran et al 1981) people with epilepsy lose more time off work than non-sufferers, and that they would rather their children did not play alongside children with epilepsy (admittedly, this latter answer may reflect a desire not to see their children's friends afflicted by epilepsy).

The continued stigma is as much as anything testament to the relative lack of efficacy and imperfections of the established agents. As will be discussed in later chapters, the treatment of seizures has advanced enormously in recent years, and in the space of around eighty years we have progressed from using glorified sedatives to using specifically designed complex antiepileptic molecules. We have entered a new era in the treatment of epilepsy.

A brief history of antiepileptic drug treatment

Even while Hippocrates was trying to rationalise the condition, a regime of drugs and dietary manipulation was formulated to treat the disease. The (then) rational treatments were placed alongside (still) superstitious remedies: the former suited for those rich enough to afford the considerable costs, the latter to give the impoverished masses a quick, inexpensive attempt at cure. In many different cultures, skull trephination was carried out in an attempt to let 'evil spirits' escape from the skull (Gross 1992).

The passage of time from ancient times to the mid eighteenth century saw no real advance in the treatment of epilepsy. Many animals were sacrificed at one time or another in an attempt to provide body parts which would abate seizure activity. An unhealthy (and rather unappetising!) interest in other species' genitals was demonstrated for their suggested pharmacological properties. Fortunately for the seals, hippopotami, hares, boars and rams (among others), the antiepileptic effect of their pudenda has not been proven, and their use is thankfully not routine in today's epilepsy clinics.

Even well into the nineteenth century, the development of rational treatments for epilepsy lagged some way behind the knowledge of epilepsy pathophysiology. Silver nitrate was commonly used in hospices around England, but it was not until the late 19th century that the first steps towards effective anticonvulsant pharmacology were made. Working on the assumption that epilepsy was a manifestation of either hysteria or sexual frustration, bromide salts were prescribed. These were a mixture of potassium, sodium and ammonium bromide, and they did, admittedly, possess

previously unprecedented anticonvulsant properties according to Sir Charles Locock's presentation to the Royal Medical Chirurgical Society in 1857 (Sieveking 1857), and by the last quarter of the 19th century, 2.5 tonnes of bromide salts were being used annually at the National Hospital for Nervous Diseases (Holmes 1954). Clouston in the introduction to his open trial of bromide salts in 1868, included an elegant plea for evidence-based medicine.

> *"What asylum physician ... has any approach to a feeling of certainty that drugs will have the effect he anticipates? ... We have statements of individual authors in regard to the right mode of giving some drugs, but after all these are merely opinions founded on most limited observations, and lack the exactitude of research , and the numerical basis on which alone scientific truth is based"*

This early study of what has transpired to be the first largely GABAergic drug showed not only a reduction in seizure frequency, but also changes in seizure morphology on treatment with up to 50 grains of bromide salts daily. In asylums round the country, bromide-induced stupor and skin abcesses were thought to be preferable to poorly controlled epilepsy, but were enough to render the bromides obsolete once a well-tolerated alternative had been discovered.

1912 saw the first use of the sedative barbiturates in epilepsy thanks to a chance observation by Hauptmann (Cereghino and Penry, 1995). Although

less sedative than bromide salts, the barbiturates are far from ideal as AEDs, but they remained the only man-made compounds in use as first-line anticonvulsants, unrivalled for almost 30 years. With the work of Putnam and Merrit on animal seizure models, the modern age of epilepsy treatment had begun.

Phenytoin is an effective anticonvulsant drug which was introduced in 1938. Phenytoin possessed previously unrivalled antiepileptic properties without being overtly sedative, and this advance in the therapy of epilepsy helped to fuel a wave of optimism. The emphasis for epilepsy care was to move from colonisation to integration. Patients progressed from being 'epileptics' to being 'people with epilepsy' and education programmes were set up to remove the stigma which accompanied the diagnosis of seizure disorder. It is testimony to its efficacy that despite its well recognised adverse event profile, phenytoin remains one of the most commonly used AEDs in many parts of the developed world, particularly the USA.

After almost 30 years of relative quiescence, following the introduction of phenytoin, the 1960's saw the introduction of carbamazepine and sodium valproate which, for the moment at least, are the two first-line antiepileptic drugs in the UK in terms of both tolerability and efficacy.

Some benzodiazepines, particularly clobazam and clonazepam, did gain in popularity in the mid-seventies, although their use was limited by the tolerance which develops even with short-term use. Detailed discussion of the benefits and drawbacks of each antiepileptic drug will be covered in the next chapter, putting the search for new anticonvulsant compounds in context.

By the late seventies, it was still apparent that more effective, more tolerable antiepileptic drugs were still needed. The increasing knowledge of the role played by amino acids in neurotransmission allowed us to search for drugs with discrete, well defined neurochemical actions. The majority of the newer AEDs are a product of this search, and we may at last be moving away from a reliance on glorified sedatives which constitute the established anticonvulsants. We can only hope that any increased specificity of the new generation of AEDs will maintain or improve anticonvulsant efficacy while avoiding the array of non-specific side effects associated with the older agents. Four new AEDs have been granted a licence for use as add-on therapy in the UK in the last 5 years, and there is an unprecedented range of compounds which are awaiting further clinical and pre-clinical trials to support their claims as effective anticonvulsants. This relatively rapid progress contrasts with the slower advances of bygone years (Figure 1), and there may be as many as eleven new AEDs on the market by the end of the century (Brodie and Dichter 1996) - giving us a potentially bewildering array of therapeutic options for patients with epilepsy.

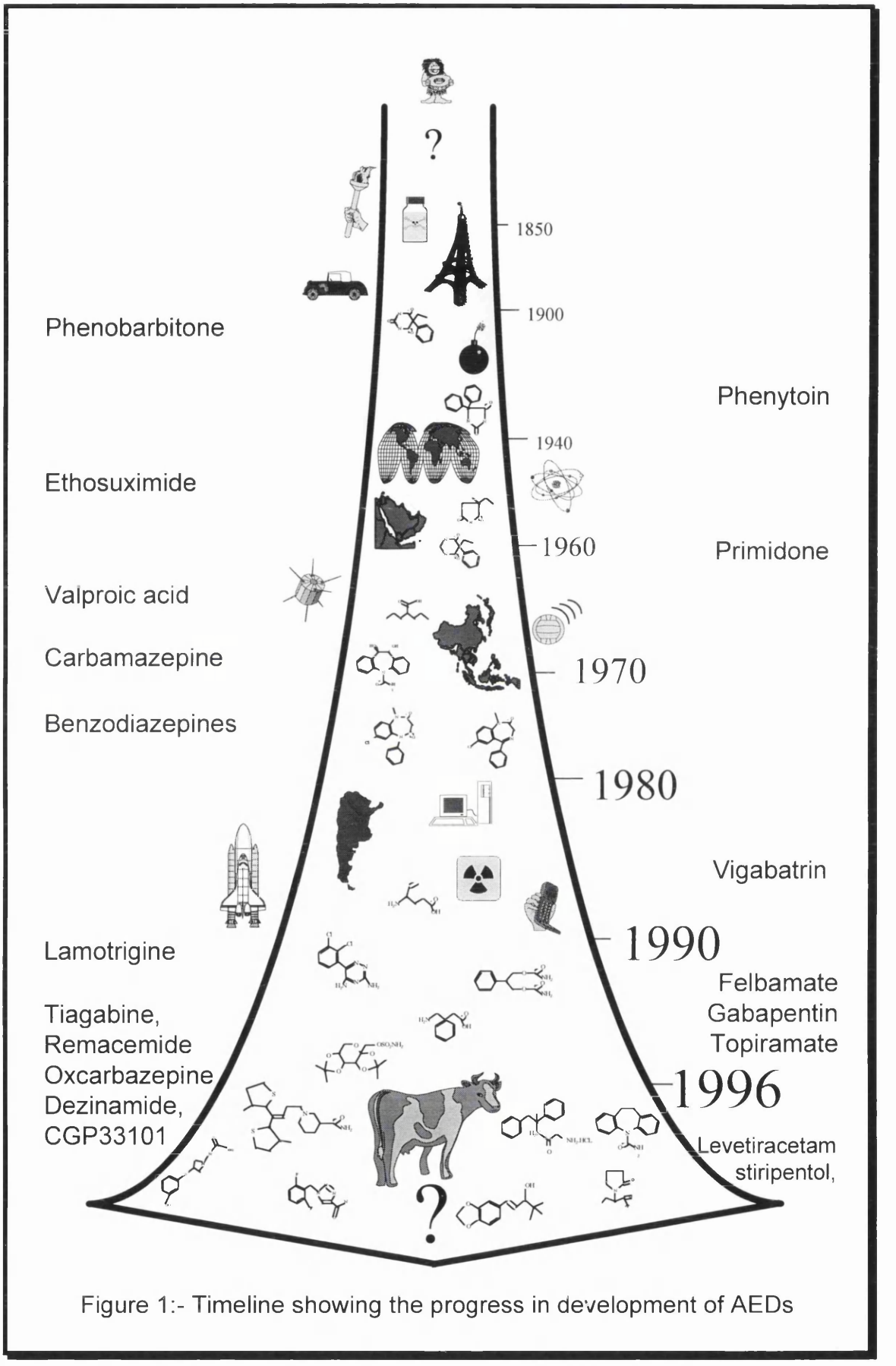


Figure 1:- Timeline showing the progress in development of AEDs

Seizure classification

It is pertinent to discuss seizure classification at this point, prior to the discussion of each anticonvulsant agent. Understanding the indications for, and the failings of, each AED depends in part on the definitions of seizure type. In 1981, the International League Against Epilepsy (ILEA) devised in a classification which relied on the symptomatology and third party witness history of seizures. Eight years later an updated classification was adopted by the ILAE (Commission on Classification and Terminology of the ILAE 1989) providing a more complex and unyielding classification of seizures which has largely superseded the 1981 version.

The 1989 version takes account of syndromic classifications and possible aetiologies, and creates a rigorous distinction between epilepsies that are idiopathic ("no predisposing cause other than an hereditary disposition"), symptomatic ("the consequence of a known or suspected disorder of the CNS"), and cryptogenic ("presumed to be symptomatic, but often lack well defined electroclinical characteristics"). Within the localisation-related epilepsies, the classification also described variants dependent on their proposed anatomical localisation. This classification was not intended to be exhaustive, and with constant updating of the technologies available for structural, electrical and metabolic imaging, our understanding of seizure morphology it will undoubtedly require constant updating.

1 Localisation-related epilepsies and syndromes

1.1 Idiopathic (with age-related onset)

- Benign Childhood epilepsy with centrotemporal spikes
- Childhood epilepsy with occipital paroxysms
- Primary reading epilepsy

1.2 Symptomatic

- Temporal lobe epilepsies
- Frontal lobe epilepsies
- Parietal lobe epilepsies
- Occipital lobe epilepsies
- Chronic progressive epilepsia partialis continua of childhood (Kojewnikow's syndrome)
- Syndromes characterised by seizures with specific modes of precipitation (eg reflex epilepsies)

1.3 Cryptogenic

- As in 1.2, but lack of aetiological evidence.

2 Generalised epilepsies and syndromes

2.1 Idiopathic (with age-related onset - listed in order of age of onset)

- Benign neonatal familial convulsions
- Benign neonatal convulsions
- Benign myoclonic epilepsy in infancy
- Childhood absence epilepsy
- Juvenile absence epilepsy
- Juvenile myoclonic epilepsy
- Epilepsy with grand mal seizure on awakening
- Other generalised epilepsies
- Epilepsies precipitated by specific modes of activation

2.2 Cryptogenic or symptomatic (in order of age of onset)

- West syndrome (infantile spasms)
- Lennox-Gastaut syndrome
- Epilepsy with myoclonic astatic seizures
- Epilepsy with myoclonic absences

2.3 Symptomatic

2.3.1 Non-specific Aetiology

- Early myoclonic encephalopathy
- Early infantile epileptic encephalopathy with suppression burst
- Other symptomatic generalised epilepsies not defined above

2.3.2 Specific syndromes

- Seizures as presentation of other diseases

3 Epilepsies and syndromes undetermined to be focal or generalised

3.1 With both focal and generalised seizures

- Neonatal seizures
- Severe myoclonic epilepsy in infancy
- Epilepsy with continuous spike-waves during SW sleep
- Acquired epileptic aphasia
- Other undetermined epilepsies not mentioned above

3.2 Without equivocal features of generalisation or focal seizure

4 Special syndromes

4.1 Situation-related seizures

- Febrile convulsions
- Isolated seizures or isolated status epilepticus
- Seizure only in the presence of an acute metabolic or toxic event

1989 ILAE Classification of seizures and syndromes

Characteristics of seizures with anatomical localisation

- **Temporal lobe epilepsies**

Temporal lobe syndromes characteristically cause a range of seizure types from simple partial (SP) seizures, complex partial (CP) seizures or secondary generalised seizures. Onset is frequently in childhood or young adulthood, and there is often a history of febrile convulsions and / or a family history of epilepsy.

SP seizures typically involve autonomic or psychic symptoms without disturbance of consciousness. These may take the form of auditory or olfactory hallucinations. An epigastric 'rising' sensation is frequently the first symptom to be reported.

CP seizures often begin with motor arrest which is quickly followed by primitive automatisms. Other more complex automatisms may follow. Attacks usually last longer than 1 minute, and recovery is gradual with post-ictal confusion and subsequent amnesia. EEG in temporal lobe epilepsy may be normal, or may show slight asymmetry of the background activity. Temporal spikes, sharp waves, or slow waves may be bilateral or unilateral, and may be better defined by intracranial recordings.

Seizures from specific areas.

Amygdalo-hippocampal seizures (mesiolabial limbic or rhinencephalic)

Hippocampal seizures are the most common, with features as described above except that auditory hallucinations are uncommon. Seizures are characterised by rising epigastric discomfort, nausea, autonomic signs and

symptoms such as belching, borborygmi, pallor, facial flushing, pupillary dilatation, fear, panic, and olfactory-gustatory hallucinations.

Lateral temporal seizures

Epileptic foci in this area causes simple seizures characterised by auditory hallucinations, illusions, or dreamy states. Visual misperceptions or language disorders may occur where the language dominant hemisphere is involved. These may progress to complex partial seizures as described above. EEG occasionally localises temporal spikes which are most prominent in the lateral derivations.

- **Frontal Lobe epilepsies.**

Epileptic foci in the frontal lobe cause one or more types of SP, CP, or secondary generalised (SGTC) seizures. Seizures are frequently nocturnal and may be easily mistaken for psychogenic seizures. Status epilepticus is more common than with other areas of abnormality.

Frontal lobe foci may be suggested when seizures are short, involve a rapid recovery, or may undergo rapid secondary generalisation. Motor manifestations are prominent and may be bizarre, with complex gestural automatisms frequent at the onset of seizure. If the discharge is bilateral, then falls are frequent. Secondary generalisation is more common than with temporal lobe foci, and tends to occur more rapidly.

Seizures from specific areas.

◊ *Supplementary motor seizures*

Patterns of this seizure type involve focal tonic seizures with vocalisation, speech arrest, and fencing postures.

◊ *Cingulate*

Cingulate seizures tend to be CP seizures with complex motor gestural automatisms at onset. Autonomic signs occur commonly as do changes in mood and affect.

◊ *Anterior frontopolar region*

Patterns in this area include forced thinking or initial loss of contact and adversive movements of head and eyes. These may evolve into contraversive movements and axial clonic jerks, with falls and autonomic signs.

◊ *Orbitofrontal*

The characteristic pattern is of CPS with initial motor and gestural and motor automatisms, olfactory hallucinations, illusions and autonomic signs.

◊ *Dorsolateral*

Usually a tonic seizure pattern, less commonly clonic seizures with versive head and eye movements and speech arrest.

◊ *Opercular*

Include mastication, salivation, swallowing, laryngeal, speech arrest, autonomic symptoms. Secondary sensory changes may consist of numbness particularly in the hands. Gustatory hallucinations are particularly common.

◊ *Motor cortex*

Mainly characterised by simple partial motor epilepsies. The nature of these will vary depending on the size and topography of the area involved. In the pre-rolandic area, there may be speech arrest, vocalisation, dysphasia, tonic

clonic movements of the contralateral facial muscles, or swallowing. Foci in the rolandic area cause partial motor seizures without march, particularly occurring in the contralateral extremities. Todd's paralysis is frequent.

In frontal lobe epilepsies, the EEG may well be normal, or may show spikes sharp waves or slow waves, unilaterally or bilaterally. Intracranial recordings may provide more information.

- **Parietal lobe epilepsies.**

Foci in this region usually cause SP and SGTC seizures. CP seizures may evolve from SP seizures with outward spread. Seizures are predominately sensory, with positive or negative phenomena which may be confined or may spread in a Jacksonian manner. The areas usually affected are those with the largest cortical representation (hand, arm, face), and there may be lingual crawling, stiffness or coldness affecting the face bilaterally. Occasionally abdominal sinking, choking or nausea may be reported, and rarely there may be sensation of pain. Parietal visual symptoms may consist of formed hallucinations and visual distortions. Inferior parietal lobe involvement may cause negative phenomena such as severe vertigo or disorientation in space. Paracentral involvement may cause genital sensations, and foci in this area have a greater tendency to secondarily generalise.

Occipital seizures

Seizures from this area are usually characterised by SP and SGTC seizures, although CP seizures may occur with outward spread. Clinically the seizures

usually involve visual manifestations which may be negative (scotoma, hemianopia, amaurosis) or more commonly positive (sparks, flashes, phosphenes). Perceptive illusions may involve a distortion of perceived distance, shape, or size of objects. Illusional and hallucinatory visual seizures involve discharges at the temporoparieto-occipital junction. Versive movements may occur. Frontal lobe seizures may be mimicked if forward spread occurs to the suprasylvian convexity or the mesial surface.

The physiological and biochemical basis of seizures

The last forty years have seen enormous advances in our understanding of neurotransmission, but despite this the basic mechanisms underlying epileptic seizures are still to be fully defined. Elucidation of the biochemical defects and effects surrounding epileptogenesis has helped to point the way towards newer more effective AEDs, and in the future will be essential in highlighting the most promising directions for development of future therapies.

Normal neurotransmitter actions

Amino acid neurotransmitters have long been recognised as being of import in the propagation and cessation of epileptic activity (Horton 1989). In terms of their role in neurotransmission, amino acids can be subdivided into those that are excitatory (glutamate, aspartate) and those that have inhibitory actions (GABA).

Normal ionic fluxes in neurones.

When an excitatory input arrives at a section of neuronal membrane, the membrane potential initially shifts from it's resting value of -70mV. If the sum of all co-incident excitatory post-synaptic potentials (EPSP) is sufficient to diminish the membrane potential to around -60mV (the 'threshold potential'), there is a resultant large increase in both Na^+ influx and K^+ efflux, which is a requirement for the generation of a propagated action potential.

Inhibitory factors can diminish the effect of an EPSP, via an increase in Cl^-

conductance which causes the membrane potential to become more negative (hyperpolarise). This hyperpolarisation is a result of what is (unsurprisingly) termed an inhibitory post synaptic potential (IPSP).

In addition to these 'fast' responses in ionic conductance, there are slower, neurotransmitter-dependent responses which occur as a result of electrical stimulation.

Gamma aminobutyric acid (GABA)

GABA concentration has been shown to be highest in the hypothalamus, globus pallidus and substantia nigra (Fahn 1976, Enna 1981). Although activation of $GABA_A$ receptors inhibits individual neurones, it has been shown that the relationship between GABA and the prevention of convulsions is far from uniform (Gale 1989, Gale 1992), and while in some brain regions localised enhancement of GABA levels may be proconvulsant (eg. pontine reticular formation), depletion of GABA in others may not affect seizure activity (eg. substantia nigra) (Gale 1992).

GABA formation and metabolism

GABA is formed by the decarboxylation of glutamate (Figure 2), a process catalysed by 2 isoforms of the enzyme glutamic acid decarboxylase (GAD) (Martin and Rimvall 1993).

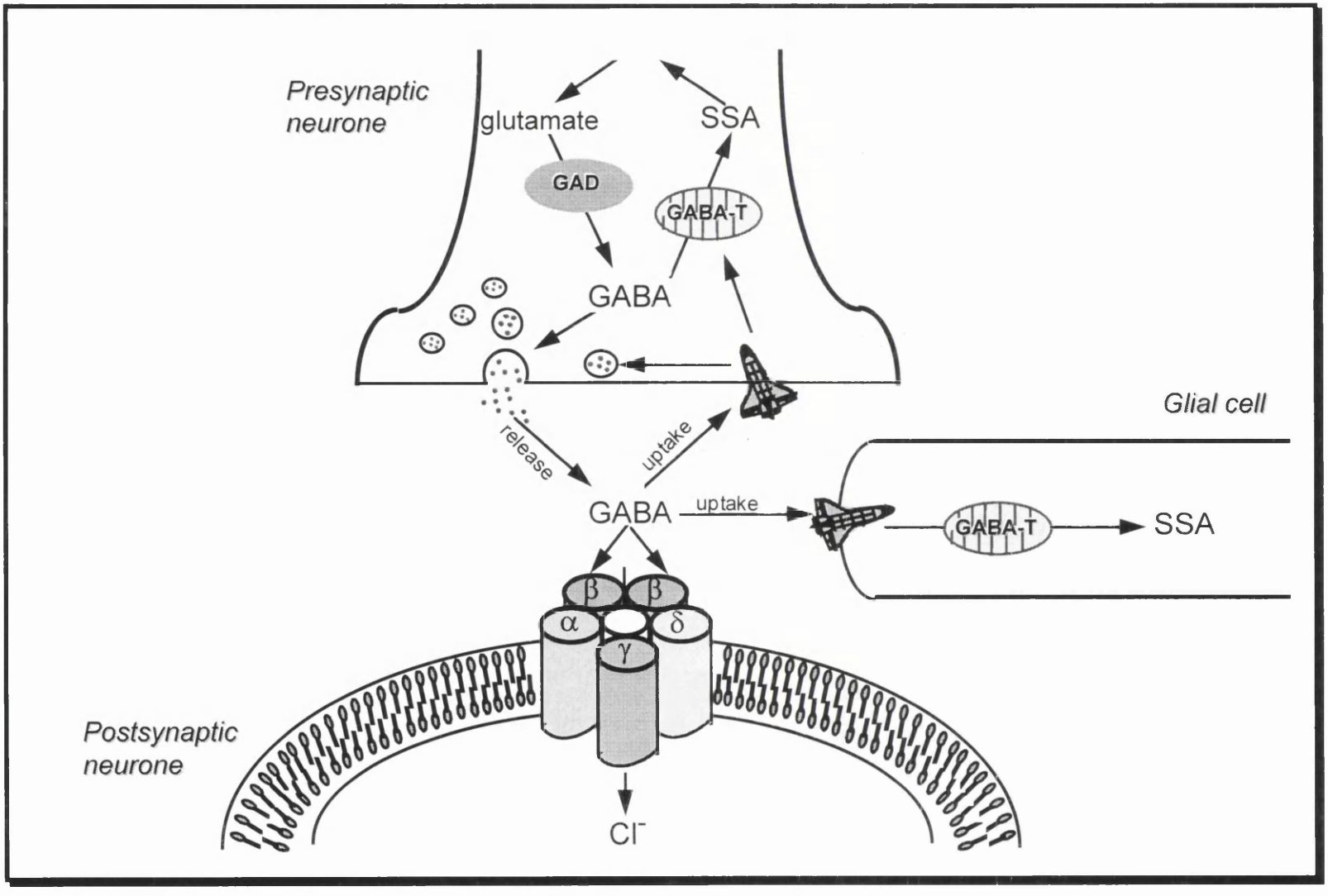


Figure 2
Metabolism and reuptake of GABA.

Other routes of synthesis are thought to be of little import in neurological tissues under normal conditions, and the immunocytochemical staining for GAD is used as a marker for GABAergic neurones (Snead 1983).

As with other decarboxylase enzymes, GAD requires the presence of a cofactor, pyridoxal phosphate, for maximal activity. Physiological changes in glutamate and adenosine nucleotides may alter enzyme activity in vivo, although in mammals, there is no feedback inhibition of GAD activity by GABA concentrations (Horton 1989).

It has been shown that GAD undergoes calcium-dependent binding to cell membranes, which is itself inhibited by Cl^-, Zn^{2+}, and sulphydril agents (Horton 1989), although the clinical significance of this is unclear. GABA release occurs in response to electrical and K^+ stimulation of GABAergic neurones (Horton 1989). This release is calcium-dependent and blocked by tetanus toxoid. An increase in extracellular GABA concentrations, or the presence of GABA agonists, inhibits further release of GABA (Horton 1989), suggesting that autoreceptors play a part in regulating the extent of GABAergic inhibition.

There are two main types of GABA receptors, $GABA_A$ and $GABA_B$ (Enna and Gallagher 1983). Activation of the $GABA_A$ receptor leads to Cl^- influx into the cell with associated hyperpolarisation and resultant diminishment of epileptic activity (Enna and Gallagher 1983).

Each $GABA_A$ receptor requires five subunits to combine, forming a chloride-selective ion channel (Olsen 1982, Levitan et al 1988, Olsen and Tobin 1990), with additional binding sites for BDZs (Ehlert 1986), barbiturates (Willow and

Johnston 1983), picrotoxin (Olsen 1981), penicillin (Twyman et al 1992), and neurosteroids (Majewska 1986). At least thirteen different $GABA_A$ receptor subunit isoforms have been described, although fewer than ten are likely to be of importance (McKernan and Whiting 1996). Many $GABA_A$ receptor subtypes are found only in selected regions of the brain (MacDonald and Kelly 1993), which may account for each drug's specificity of action.

Less is known about the $GABA_B$ receptor. It was initially thought to be purely a presynaptic autoreceptor, mediating negative feedback of GABA release from the presynaptic neurone. It is now clear that $GABA_B$ agonism has other effects and it's physiological role is less clear (Enna and Gallagher 1983).

GABA uptake is undertaken by a structurally-specific high affinity active transport mechanism which is present in both astrocytes and neurones (Iversen and Kelly 1975) presumably to limit the inhibition mediated by synaptic GABA. There are four GABA transport structures described (Borden et al 1994), one of which (GAT-1) would appear to be the main site of action of known uptake inhibitors such as nipecotic acid. The derivatives also demonstrate a lesser inhibition of uptake of GABA by the GAT-3 transporter mechanism (Clark and Amara 1994). The transport mechanism is temperature-dependent, and requires the presence of extracellular Na^+ (Borden et al 1994). There is a recognised high correlation between GAD activity and the rate of GABA uptake, suggesting that, for neurones at least, the extent of GABA uptake is related to the GABAergic activity of the cell (Horton 1989).

Once inside the cell, GABA is broken down (Meldrum 1975) by GABA-

transaminase (GABA-T) to succinic semialdehyde (SSA). Subsequent breakdown of SSA occurs via the dehydrogenase enzyme (SSADH), a reaction that is preferentially inhibited by sodium valproate (Harvey et al 1975).

Glycine

Glycine is probably of most importance in inhibiting neuronal firing at the level of the spinal cord and brainstem. It's concentration in grey matter is uniformly low, although glycine-mediated inhibition has been reported in some higher brain centres, such as the substantia nigra, cerebellum and cortex.

Like GABA, it is known to cause hyperpolarisation via an increase in Cl^- conductance (Horton 1989), although this effect is mediated by different receptors. Glycine is metabolised to serine by serine hydroxymethyltransferase (SHMT), and also to glycoxylate by glycine aminotransferase. Glycine receptors are selectively antagonised by strychnine and other related alkaloids (Horton 1989).

Excitatory Amino Acids

Glutamate and Aspartate

These two dicarboxylic acids have similar kinetics, undergo mutual inhibition, and are inhibited by the same structural analogues, indicating that they are probably taken up by the same carrier (Figure 3). The metabolism of both is tied up with the intermediaries of the tricarboxylic acid cycle: aspartate can be formed by transamination of oxaloacetate or from CO_2 fixation with pyruvate.

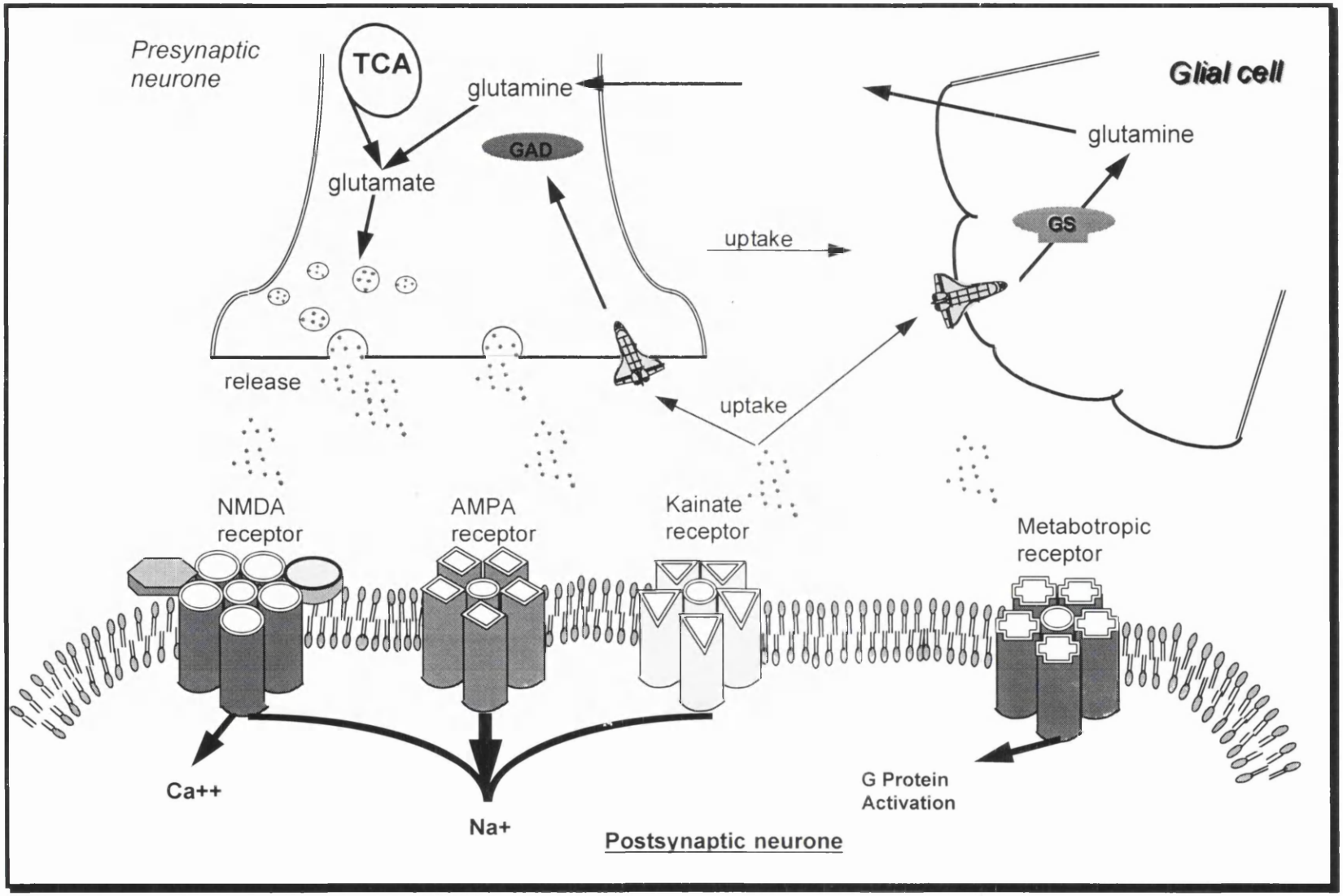


Figure 3
The metabolism, reuptake and post-synaptic receptors of glutamate

Most of the glutamate available as neurotransmitter is derived from neuronal de-amination of glutamine, but it can, however, also be formed by the transamination of α-oxoglutarate, or direct amination of oxoglutarate by ammonia (Peng et al 1991). Like GABA, glutamate is released by electrical or potassium-induced depolarisation. This release is Ca^{2+}-dependent, and is inhibited by Mg^{2+} (Horton 1989, Nicholls and Attwell 1990).

Glutamate receptors may be divided into those that are ionotrophic and those that are metabotropic (Watson and Girdlestone 1995). The ionotrophic glutamate receptors are further divided by their selective agonists, NMDA, kainate and AMPA (Monaghan et al 1989). Activation of these receptors is thought to make the channels permeable to Na^+, K^+ and (in the case of the NMDA subtype), Ca^{2+} (Mayer and Miller 1990). The resultant Na^+ influx, Ca^{2+} influx, or K^+ efflux is thought to be the basic mechanism by which glutamate effects cell death (Watkins and Evans 1981). The metabotropic glutamate receptors are G-protein linked (Schoepp et al 1990), and there are currently thought to be at least 7 subtypes of this ($mGlu_1$-$mGlu_7$) (Watson and Gridlestone 1995).

Both glutamate and aspartate are removed from the synapse into glial cells and neuronal dendrite endings via a high affinity, sodium dependent uptake system (Drejer et al 1982). In glial cells, glutamate is converted to glutamine, via glutamine synthase (GS) which is then transported out of the astrocyte and taken up by neurones, and again de-aminated to form glutamate: the so-called glutamine cycle (Watkins and Evans 1981).

Abnormalities demonstrable in epileptic brain tissue

The exact delineation of the cause and effects of epileptic activity on neurones' structure and function has still to be carried out. The picture is still confused, with no real cohesion between proponents of various theories involving disturbance of neuronal networks, altered amino acid metabolism, or genetic membrane abnormalities.

It is sensible to assume that different seizure types have, at their root, different biochemical and pathological changes, and for this reason, these seizure types will be considered separately. Gloor and Fariello (1988) in their work on feline generalised epilepsy were supportive of the notion that the underlying abnormalities in primary generalised epilepsies were different from those in focal seizures.

The Focal Epilepsies

Focal seizures are currently better understood than generalised seizures, and in localisation-related seizures there are thought to be two different primary cellular events, which result in the abnormal activity that produces either ictal or interictal discharges (Dingledine et al 1990, Meldrum 1988). The distinction between the two types of discharges is made, by definition, on whether it is sustained enough or severe enough to produce any clinical sequelae.

Burst firing in single neurones is the basic electrical event underlying the appearance of interictal discharges on EEG, having been observed in acute *in vitro* models, as well as in chronic seizure foci in animals and man (Meyer et al 1986). The cellular mechanisms responsible for these episodes are

discussed below. During interictal discharges, intracellular recording has shown there to be a paroxysmal depolarising shift (PDS) in the resting membrane potential, consisting of a short but sustained period of depolarisation, which facilitates a burst of action potentials (Ayala et al 1970). Inhibitory interneurone input (Meldrum 1988), then brings about a phase of hyperpolarisation which helps to prevent the rapid re-occurrence of a further PDS. The PDS in each individual neurone contributes to the extracellular field potential which manifests as the interictal spike on surface EEG tracings (Matsumoto and Ajmone-Marsan 1964).

Horizontal spread of these interictal events within the same class of neurones can result in the progression from involvement of a single cell to multiple burst firing within the same neuronal aggregate (Meyer et al 1986). Inhibitory mechanisms are responsible for limiting the space and time over which this recruitment can continue (Meldrum 1988). These inhibitory mechanisms will certainly be synapse mediated (Traub et al 1987), and may also involve gap junctions between neurones, extraneuronal ionic changes, and ephaptic spread (Dichter and Ayala 1987).

Interictal firing spreads to a variable degree, but the rate of spread increases as the abnormal events become more intense and rhythmically recurrent (Meldrum 1988). The strength of local inhibitory control mechanisms is an important controlling factor determining the rate and extent of any spread.

In projection areas, during this initial activity, there is enhanced inhibitory tone which inevitably fades with repetition. Where seizure activity occurs, excitatory neurotransmission is predominant, resulting in synchronised burst

firing in subsequent synaptically-linked neuronal aggregates (Meldrum 1990). Collingridge and Singer (1990) asserted that this spread relies on the phenomenon of frequency-dependent inhibitory fade.

Where discharges fail to progress to a stage where clinical seizure activity occurs, then the event is an interictal discharge. The termination phase of this episode involves the onset of sustained hyperpolarisation (Meldrum 1990), mediated by inhibitory processes. Why only a proportion of interictal discharges progress to frank seizure activity is, like many aspects of basic epileptogenesis, a mystery, but it may be the result of frequency-dependent depression of local inhibition (Prince 1985).

Disinhibition has two results. Not only will the interictal discharge be propagated to the neuronal aggregate, but there is a marked reduction in the duration of the hyperpolarisation phase. Increased excitation and decreased inhibition combine to give perfect conditions for an increase in frequency of interictal discharges *and* an increase in their propagation. This situation has been likened to a fusion of successive PDSs with resultant lack in any intervening repolarisation (Meldrum 1988). Ayala et al (1970) characterised the prolonged depolarisations with accompanying bursts of action potentials. With accompanying disinhibition, this abnormal electrical activity will be sufficiently sustained to propagate to the descending motor tracts, resulting in clinical seizure activity.

Burst firing, as defined above, could occur in several ways. It may either be the result of activity in an abnormal 'epileptic' neurone, or may occur in a normal neurone either as a response to abnormal input at the nerve terminal-

dendrite interface, or secondary to an alteration in the local ionic micro-environment (Meldrum 1989).

Other theories on the basic mechanisms of epileptogenesis include, as a prerequisite, some event causing damage to the affected brain tissue. This event may be 'subclinical' such as minor head injury, or neonatal hypoxia, and may excite no clinical interest at the time of occurrence. Pathological examination of epileptic foci in human brain has shown some underlying neurodegeneration (Schwartzkroin 1994), and this may be a feature of all secondary epilepsies. Meldrum (1989) described the appearance of histological changes arising from cellular microdysgenesis, congenital vascular malformations, neoplasms, ischaemia / infarction, traumatic brain lesions, infarcts, abscesses, and cysts. Also implicated was the diffuse degeneration associated with diverse neurological disorders, such as Huntington's chorea and Alzheimer's disease

Whether localised injury preferentially affects GABAergic neurones is a point of some debate. There is evidence in animals to suggest that, in infancy, hypoxia can selectively damage cortical GABA neurones (Sloper et al 1980). Ribak et al (1979) showed that local application of alumina gel to cerebral cortex in monkeys prompted the appearance of an epileptic focus which demonstrated a selective loss of GABAergic nerve terminals. Babb and colleagues (1989) on the other hand, showed that neurones staining for glutamic acid decarboxylase (GAD) were not selectively affected in human epileptic hippocampus.

Ischaemic lesions of the spinal cord have been related to the selective loss of

inhibitory glycinergic interneurones (Meldrum 1989), a decrease in inhibition and resultant spasticity. Potential mediators of this specific cell loss have been associated with cellular microdysgenesis, ischaemia / infarction, traumatic injury, and focal neoplasia. Meldrum (1989), however, felt there was an equal reduction in both excitatory and inhibitory neurones from epileptic foci.

Whatever the cause of loss of GABAergic neurones, when their number or function is impaired, the balance tips towards excitation, and other neurones are predisposed to burst firing (Miles and Wong 1987). When GABAergic interneurones can induce adequate hyperpolarisation, the soma is well protected against invasion by burst firing and PDSs from the nerve terminal dendritic interface (Meldrum 1989). Where GABAergic neurones have incomplete input, the reduced hyperpolarisation is not enough to resist electrical changes, and somal recordings will show evidence of PDSs and burst firing (Schwartzkroin and Wyler 1980). The loss of excitatory neurones may be a direct result of seizure activity, as has been shown in electrically kindled seizures in animals (Sutula 1990).

Is it possible that a seizure-induced alteration in neuronal glutamate sensitivity may also predispose to epileptic activity? Meldrum (1989) described secondary phenomena occurring with cell loss induced by local neuronal degeneration which may increase the predisposition to spontaneous neuronal discharge. Neurones which are postsynaptic to the degenerating cells 'upgrade' their receptors and show supersensitivity to the neurotransmitter which is lacking. The hippocampus of kindled rats will

demonstrate this under experimental conditions. Kindling-induced loss of excitatory pyramidal neurones is followed by supersensitivity of CA1 dendritic zones and the dentate molecular layer to excitatory amino acid (EAA) neurotransmitters such as aspartate and glutamate (Mody and Heineman 1987). Interestingly, Louvel et al (1992) found an alteration in responsivity to NMDA in slices of neocortices from patients with epilepsy, a finding that would be consistent with this hypothesis. This may predispose to an exaggerated response to normal extracellular concentrations of EAA, perhaps sufficient to precipitate burst firing. There is further evidence to support this theory, where neocortex which has been deafferented by undercutting shows an enhanced tendency to display epileptiform "afterdischarges" following electrical stimulation (Meldrum 1989). Some animal models suggested that the development of this supersensitivity develops over a similar length of time as can be taken to develop focal epilepsy. In patients developing epilepsy secondary to focal pathology, however, the time course is variable and often much longer than the time required to upgrade receptors (Meldrum 1989).

Where synaptic degeneration occurs, adjacent dendrites can 'sprout' and grow to form new synapses in the vacant spaces. These synapses can provide inappropriate input (Meldrum 1989), as discussed below. Neuronal degeneration will inevitably lead to some degree of sprouting and supersensitivity, and the combination may provide the basis for all symptomatic epilepsies (Meldrum 1989).

The use of silver staining techniques shows neuronal changes which are universally seen in histologically examined epileptogenic tissue. The

architecture of the hippocampus and neocortex becomes simplified. There is a progressive loss of the complex dendritic trees, with a loss of spinous processes. The reasons for this degeneration are unclear, although it may be at least in part due to supersensitivity to n-methyl-d-aspartate (NMDA), as is observed in electrophysiological studies (Meldrum 1989). Such simplification has been observed experimentally, in the region of an alumina-induced epileptic focus in the monkey cortex (Ribak et al 1979). How this can occur alongside the occurrence of sprouting is not fully understood, and it should be said that sprouting is a process which is not specific to epilepsy, having been reported in a wide range of other neurodegenerative disorders (Meldrum 1989).

Simplification of epileptic tissue may correlate with an enhanced tendency to develop PDSs, either via an increased instability of the degenerating neurone, or an increased susceptibility of the soma to invasion due to electrotonic shortening (Meldrum 1989).

The role of glial cells in the development of epilepsy

Glial cells are the predominant cell type in the CNS, outnumbering neurones by a factor of ten in mammalian brain (Kimelberg 1983). Around half of all glial cells are astrocytes, and these are now subdivided into type I and type II astrocytes. Traditionally, as the name suggested, it was believed that glial cells were the glue in the CNS, being a sophisticated scaffolding around which neurones are draped. At their most sophisticated, astrocytes were thought to act out a 'housekeeping' role (Montgomery 1994). The evolution of

astrocyte culture techniques has allowed selective culture of type I and type II astrocytes, and thereby permit differentiation of their physiological function.

Type I astrocytes are the more common, and among other functions, are involved in potassium homeostasis. They are known to accumulate amino acid transmitters, express receptors for a multitude of neurotransmitters, and play a role in calcium signalling (Juurlink and Hertz 1992).

Type II astrocytes, by contrast, demonstrate many properties that are traditionally 'neuronal' in nature. They have been shown to synthesise and take up GABA, and there is evidence that they may release GABA in response to glutamatergic stimulation. They possess intense basal glutamine synthase activity which may signal that they play a major role in nitrogen homeostasis and / or neurotransmitter inactivation (Juurlink and Hertz 1992).

In some brain areas, astrocytes comprise up to half of the total tissue volume (Kimelberg 1983), and given the complexity of their role (Montgomery 1994), and the frequency with which they appear in the CNS, it would seem reasonable to assume that astrocytes play some part in the development of epilepsy.

One long-observed characteristic of epileptic pathology is a localised increase in the number of fibrous or reactive astrocytes, or gliosis (Meldrum 1990). Gliosis is seen in epileptogenic foci and in diffuse degenerative disorders, and is known to develop secondary to seizure activity. It has been suggested that these reactive astrocytes may be functionally inept, and that they may fail to carry out the normal protective 'housekeeping' duties of the functional astrocyte. Walz (1989) discussed the capacity of the astrocyte to act as a

"potassium sink", postulating that any impairment of this capacity could theoretically contribute to spontaneous neuronal discharge. Studies of astrocyte function in epileptic foci, however, have actually demonstrated an *enhanced* ability to regulate extracellular potassium concentrations (Meldrum 1989), so the role of gliosis in basic epileptogenesis remains to be evaluated. The role of the astrocyte in maintaining and perhaps initiating neuronal output is becoming clearer (Juurlink and Hertz 1992). As further work is carried out on astrocyte subpopulations, they may be found to have an increasingly important part to play in initiation and limitation of seizure activity.

Changing neuronal networks and seizure propagation

One further approach to the rationalisation of localisation-related seizures involves development of theories in changes to neuronal networks. Since the days of Gowers, it has been known that seizures beget seizures. Clinical experience would suggest that the more epileptic activity that a patient experiences, the more chance there is that further seizure activity will occur in the future.

Why this should happen is not entirely clear, but two recent histologically-based theories have been formed (Shin and McNamara 1994) which may explain this tendency.

The normal function of neuronal networks is shown in diagrammatic form below. 'G' represents the cerebellar granule cell which receives excitatory input from the dendrites above. When 'G' depolarises, this exerts positive effects on the basket cell ('B') both directly and indirectly via the mossy cell

('M'). The basket cell produces inhibitory effects on 'G' which causes a hyperpolarisation, inhibiting further depolarisation as part of a negative feedback loop. In animals with epilepsy, it is known that continued excitotoxic stimulation causes degeneration of memory cells, and it is thought that this feedback is disrupted in one of two ways (see Figure 5).

1) The dormant basket cell theory

Seizure induced death of 'M' leads to a dormancy of basket cells. Any excitatory activity by 'G' therefore fails to provoke inhibitory input from 'B', and leaving 'G' more susceptible to continued depolarisation following excitatory input from synapses.

2) The Mossy Fibre Sprouting Theory

As a response to the loss of 'M' by exposure to exitotoxins, the mossy fibres from 'G' sprout and synapse to replace 'M's input to 'G's dendrites. This positive feedback loop, also lacking the excitation of inhibitory basket cells, leads to a predisposition towards excessive epileptic activity. Masakuwa et al (1992) suggested that the degree of excitability of human hippocampal slices is directly correlated with the extent of mossy fibre sprouting seen histologically.

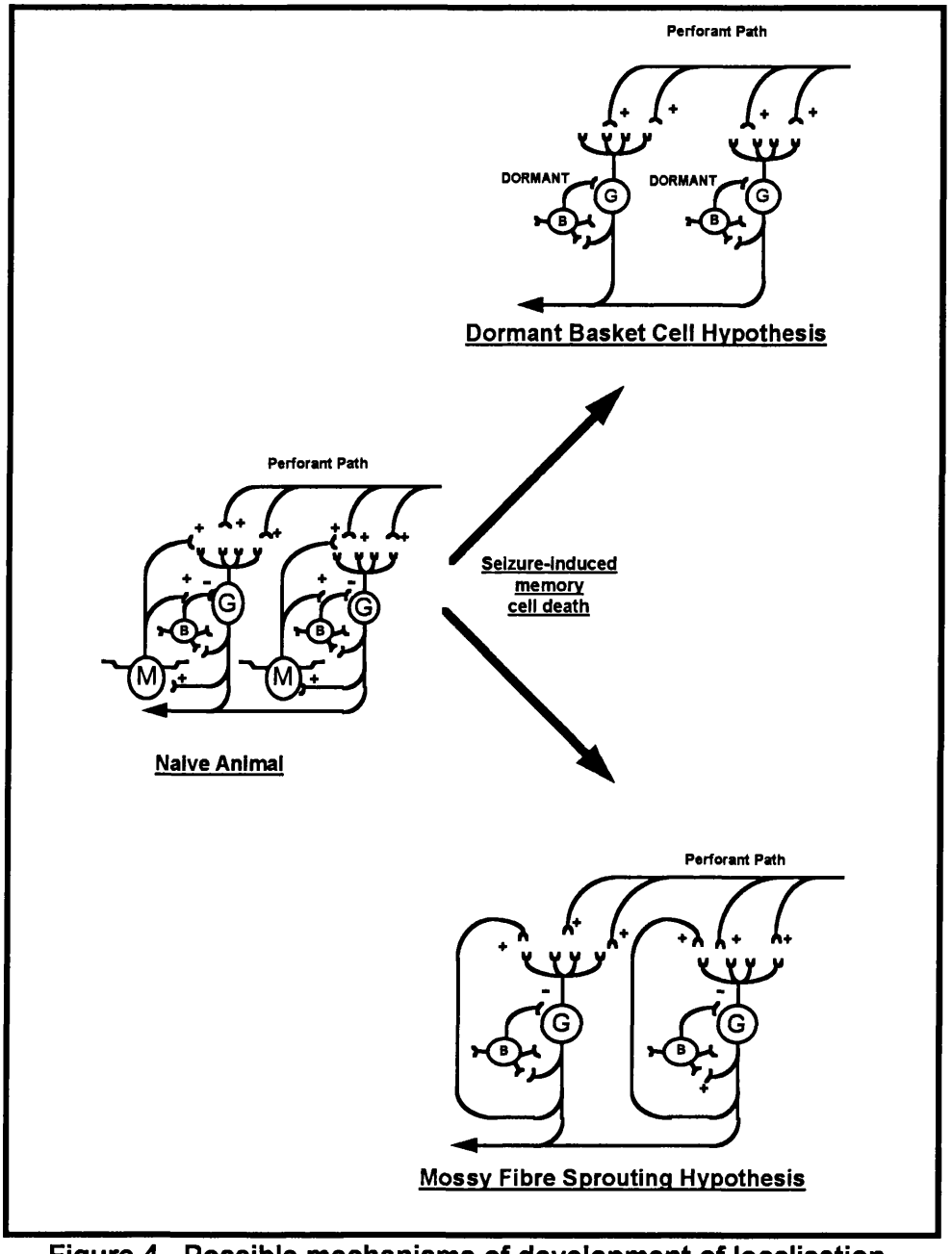


Figure 4 - Possible mechanisms of development of localisation-related epilepsy

The idiopathic generalised epilepsies

Despite some earlier expectations, we have not yet found a single molecular defect which can on its own account for the development of the primary generalised epilepsies (PGEs). This section will deal with the evidence of specifically disordered neuronal structure and function related to PGE in animal models and in their human counterparts.

Given the pattern of distribution of animal seizure models such as the audiogenic seizures or the GAERS, and allowing for the strong family histories which occur in many cases of PGE, it would be fair to assume that any defects which lead to PGE are genetic in origin. The progress in elucidation of specific genetic abnormalities which correlate with clinically well-defined epilepsy syndromes adds further weight to this assumption.

The pathological processes have been best demonstrated in the absence epilepsies, where the 'spikes' of the characteristic rhythmic synchronised activity on surface EEG represent excitatory postsynaptic potentials superimposed on action potentials, and the 'waves' represent prolonged inhibitory postsynaptic potentials (Gloor and Fariello 1988). In this form of primary generalised seizure at least, therefore, inhibitory processes must by definition be intact, so the simplistic notions of epilepsy arising from excesses of excitation or paucity of inhibition which serve us well in explaining localisation-related epilepsies, must (unfortunately!) be laid to rest.

Evidence for qualitative changes in neuronal tissue in the primary generalised epilepsies.

Samples of brain tissue prone to epileptic activity have been shown to manifest defects in adenosine triphosphate (ATP) hydrolysis mechanisms which drive the cell membrane ionic pumps (Grisar et al 1992). In DBA/2 mice, a species prone to audiogenically induced seizures, both Na^+-K^+ dependent and Ca^{2+}-activated ATPase systems were reduced in neuronal membranes (Palayoor et al 1986). Rosenblatt et al (1977) confirmed the presence of abnormalities in membrane ATPases in strains of other species such as chicks and gerbils prone to induced seizures. Meldrum (1989) and Grisar and Delgado-Escueta (1986) described abnormalities in Na^+-K^+ and Ca^{2+}-dependent ATPases in human epileptogenic tissue. Significantly, however, reductions in Na^+-K^+-dependent ATPase have been shown even where the seizure focus has been induced by experimental work, raising the possibility that changes in ATP-ase activity may be a secondary phenomenon. Other alterations have been described in genetic models of epilepsy. Age-dependent changes in density of $GABA_A$ subunit labelling have been found in the GAERS strain of rats by autoradiography (Spreafico et al 1993), with no demonstrable changes in $GABA_B$ receptor density. Significantly, this decrease in the distribution of B_2-B_3 $GABA_A$-R subunits is demonstrable only at the age where absence epilepsy becomes apparent, again raising the possibility that $GABA_A$-R expression is altered by seizure activity.

GAERS also have alterations in glutamatergic transmission, demonstrating less sensitivity to glutamate receptor blockade by CNQX than a control population (Pumain et al 1992). Staining of glutamate receptor subunits, again in GAERS, showed a decrease in proportion of GLU-R_2 and GLU-R_3 subunits

compared to control rats (Avanzini et al 1996) underlying these qualitative changes.

To understand how changes in calcium channels may predispose to the seizure generations, the role of calcium currents in normal thalamic neurones has to be defined. Different types of thalamic neurones play different roles in thalamic function. Curtis and Avanzini (1994) summarised the roles played by thalamocortical relay (TCR) neurones, local circuit interneurones (LCI), and neurones that form the reticularis thalami (RT neurones).

Chief among the differences between TCR, RT and LCI neurones is a variation in voltage-dependent ion channels (Curtis and Avanzini 1994) which, at least in part, control neuronal firing. Curtis and Avanzini (1994) described five key voltage-dependent currents: the inward T-type calcium channel (I_T), the outward calcium-activated cationic channels (I_C, I_{ahp}, and I_{CAN}), and the hyperpolarisation-activated cationic current (I_h). When the thalamic neuronal membrane potential is depolarised by neuromodulatory activity, I_T is inactivated, and the single spiking, relay mode of activity is promoted. Hyperpolarisation, however, de-inactivates I_T channels, producing a burst of action potentials superimposed on a low-threshold calcium spike. This depolarisation inactivates I_h channels, while the associated calcium entry promotes the activation of Ca^+-K^+ currents. These events lead to a $GABA_B$-mediated hyperpolarising overshoot which is suitable for reactivation of the I_T current, i.e. conditions suitable for a repeating pattern. This cycle can be broken by, for example, acetylcholine or monoamines which shift the membrane potential away from that required for reciprocal interactions, and

into a range which leads to the tonic firing mode.

On comparison with control populations, qualitative changes have been described in thalamic calcium channel activation of GAERS. Thalamic T-type Calcium currents (I_T) are specifically increased in GAERS (Curtis and Avanzini 1994), which may predispose to the rhythmic oscillatory activity described above. The dysfunction of calcium channels leading to oscillatory thalamic activity is a reasonably attainable target for putatively anticonvulsant drugs, assuming that the actions are specific enough. Certainly the anticonvulsant actions of at least one drug, ethosuximide, are probably due to a blockade of T-type calcium channels (Dichter and Brodie 1996).

Evidence for changes in neuronal interaction in generalised epilepsies

While basic properties of neuronal cell membranes are known to be altered in some seizure types, the site of the neuronal changes is also important for the development of a primary generalised epilepsies. Because of its functional connections, the thalamus is known to be important in primary generalised epilepsies. Why should a disorder of thalamic function cause such generalised regularised pathological behaviour?

As defined above, TCR and RT neurones are driven by inputs from excitatory synapses. In addition, TCR neurones are inhibited by RT and LCI neurones. LCI neurones are responsible for local feedback and feed-forward circuits within the thalamus, while RT neurones exert a more diffuse generalised inhibition, whose extent varies with the functional state of activation of the thalamus. Activation of RT neurones, therefore will lead to an inhibition of,

among others, TCR neurones.

Given the connections described, bursting behaviour in TCR neurones will therefore lead to excitation of, and subsequent inhibition from the RT system in a cyclical pattern which could account for the characteristic synchronous EEG changes.

The role of LCI neurones in synchronisation of thalamic activity is less well understood, and they are known not to be essential in the maintenance of spindle rhythm generation. The function of LCI neurones may be to control TCR neurone excitability during the relay mode (Curtis and Avanzini 1994).

The state of activation of the thalamus both influences and is dependent on the activity of the RT neurones. The sleeping, oscillatory mode of activation is associated with rhythmic burst firing, while the relay mode (where the thalamus is processing sensory information) is associated with tonic firing, or a desynchronised state. Any switch from relay to oscillatory function, and vice versa, depends on alterations in membrane and synaptic properties of the thalamic neurone. These changes come about as a result of intrathalamic and cortico-thalamic interactions and by the influence of brainstem catecholamines and cortical afferents (Curtis and Avanzini 1994). That the physiology of normal sleep associated changes should be so closely linked with the pathophysiology of generalised seizures would come as no surprise to the doctors of the nineteenth century who were quick to spot an association between fatigue or sleep deprivation and seizures. Recently Niedermyer (1996) among others has stressed the importance of arousal and sleep deprivation on all forms of PGE and their electrophysiological counterparts.

Summary

The development of PGE is not a simple process which can be ascribed to the deviant action of one simple molecular or membranous defect, or even a defect at one specific site. Each clinical manifestation of PGE will probably prove to be due to a number of factors, including multiple gene defects which lead to the gross changes of increased neuronal excitability.

A multifactorial basis for seizures may play an important role in determining our therapeutic approach to the generalised epilepsies. Given the degree of complexity, simply decreasing the effects of excitatory neurotransmission will not in itself prove sufficient. Likewise, this multifactorial aetiology will go some way to explaining why an increase in GABAergic tone may merely aggravate primary generalised epilepsies in both human (Michelucci and Tassinari 1989) and animal models (Coenen et al 1995). The occasional AED-related increase in seizures (observed at some point with almost every known AED) may prove to be a result of the multiplicity of actions of each of these complicated compounds.

This wide range of pathological processes involved in epileptogenesis should at least reinforce the concept of carefully examining the effects of all new AEDs on every potential convulsant and anticonvulsant mechanism. An anticonvulsant effect at one site is no guarantee against convulsant actions at another. Only through further work to elucidate the genesis of partial and generalised epilepsies, and to fully assess the effects of each AED will we be able to accurately predict the reaction of individuals to specific AEDs.

Is disturbed amino acid neurotransmission the root cause of epilepsy?

Evidence for the role of decreased inhibition as a cause of epilepsy

- Epileptogenic foci in primates show a selective loss of GABAergic inhibitory terminals (Ribak et al 1979), implying some local deficit in inhibitory activity.
- In tissue resected from humans there is a demonstrable decrease in GAD activity alongside increased GABA-T activity (Lloyd et al 1985), and a decrease in binding to the GABA\benzodiazepine receptor complex (Savic et al 1988).
- CSF GABA levels are reduced in patients with chronic epilepsy (Maynham et al 1980).
- GABAergic impairment is the basis for many chemically-induced animal seizure models (Fisher 1989).
- Increase in GABA concentrations or GABAergic actions is likely to be an important action of some anticonvulsant compounds (vigabatrin, tiagabine, valproate) (Schachter 1995).

Evidence for the role of increased excitation as a cause of epilepsy

- Glutamate is known to be epileptogenic when applied directly to mammalian brain (Stone and Javid 1983), or when given systemically (Bradford and Dodd 1975).
- Kindling (an animal model of epilepsy) is dependent on the presence of glutamate (Sutula 1990)

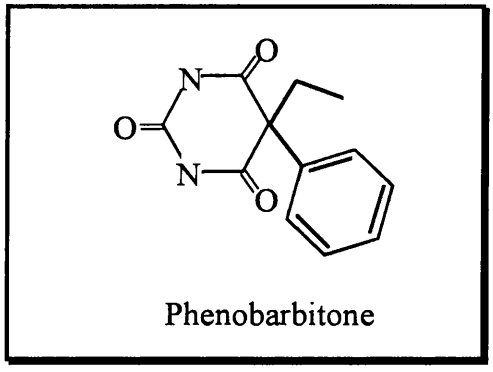
- An increase in glutamate receptor density has been demonstrated In children with generalised seizures, (Represa et al 1989), and similar changes have been shown in adults with temporal lobe epilepsy (Geddes et al 1990).
- In patients with generalised epilepsy, and in their first degree relatives, plasma glutamate has been shown to be increased (Janjua et al 1992).
- In animals (Koyama 1972) and in man (van Gelder et al 1972), there is decreased glutamate concentration within epileptic foci, which may explain glutamate receptor upregulation.

Antiepileptic Drug Treatment

The Established Anticonvulsants

As this thesis nears completion, lamotrigine, and to a lesser extent vigabatrin, are in some centres considered to have attained the status of 'established' anticonvulsant drugs. For the purposes of this thesis, the word 'established' will be used to describe those drugs which were licensed for use prior to the late 1980's. Before we dwell on the qualities of the newer agents, it is appropriate to summarise the qualities of the older agents at this point.

Phenobarbitone



Phenobarbitone

Phenobarbitone was first widely used as a sedative drug, its anticonvulsant efficacy being discovered serendipitously. First proven to be effective in 1912 by Loewe et al (Gallagher and Freer 1985), it is one of the family of barbiturate compounds that have been synthesised as part of the search for the ideal anticonvulsant (Pritchard and Ransom 1995). With the advent of better AEDs, the present day use of barbiturates in this country is more usually as a remnant of longstanding AED regimes.

Barbiturates have been shown to have a wide range of effects on many neurobiological systems (Prichard and Ransom 1995). They have a specific binding site on the $GABA_A$ receptor, the binding of which increases the frequency of chloride channel opening for a given exposure to GABA

(MacDonald and Olsen 1994). Phenobarbitone has been shown to be effective against partial and generalised tonic-clonic seizures, as well as in prevention of febrile seizures and treatment of some cases of status epilepticus (Painter and Gauss 1995).

The continued use of this landmark in AED development has suffered because of its sedative effects (Schmidt 1985), although paradoxically hyperactivity and irritability are more important adverse effects in paediatric practice (Consensus Statement 1980). The long term irreversible effects of these symptoms are not clear (Guest et al 1970).

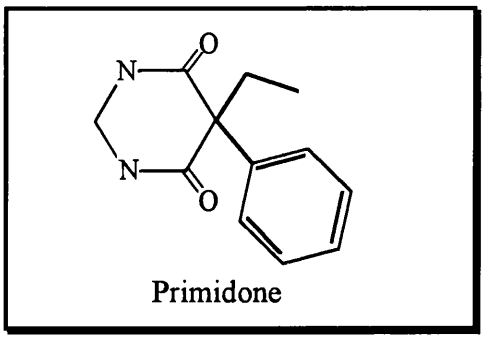
Another drawback of phenobarbitone is that, unusual among anticonvulsant drugs, withdrawal can induce further seizures (Butchal et al 1968), while development of tolerance can also prove to be a problem (Gallagher and Freer 1985). Patients who remain well controlled on phenobarbitone should not have their treatment altered unless there is good reason, because of the danger of withdrawal seizures.

Phenobarbitone is a very cheap anticonvulsant, and will remain popular in developing countries for that reason. In the developed world, where cost is less of a determining factor, it will remain less attractive than its better tolerated descendants.

Primidone

Although it has anticonvulsant activity, primidone has now ceased to be used as a first line anticonvulsant agent, following the large controlled trial (Mattson et al 1985) which showed it to be less effective and less well tolerated than

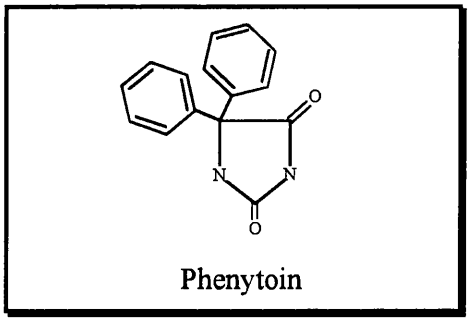
carbamazepine, phenytoin or phenobarbitone.



Primidone

The main side effects leading to withdrawal were drowsiness, gastro-intestinal intolerance and psychosis. Physical dependence (Anonymous 1992) and, in common with phenobarbitone, withdrawal seizures (Norton 1970) are also barriers to long term use. Aside from those patients who are already well controlled on primidone, it is widely recognised that this drug has no role to play in the formulation of modern anticonvulsant regimes (Brodie 1990).

Phenytoin



Phenytoin

Phenytoin entered the clinical arena in 1938 (Merrit and Putnam, 1938), and is still considered to be a first line anticonvulsant, especially in the USA (Brodie and Dichter 1996). Its activity against partial seizures, until then very resistant to treatment, was one of it's main advantages over phenobarbitone (Jones and Wimbish 1985). Phenytoin was rightly considered a breakthrough in AED development because, unlike its predecessors, the antiepileptic properties it displayed outstripped its sedative qualities. More sophisticated psychomotor testing however, has confirmed that in both patients and volunteers, phenytoin causes a deleterious effect on

psychomotor and cognitive processing (Thomson and Trimble 1981, Andrewes et al 1986, Gilham et al 1990). In some patients with a degree of learning disability, there is an increase in intellectual function (Goldberg and Kurland 1970), although this may be secondary to improved seizure control.

Phenytoin is one of the most extensively studied AEDs, and laboratory studies have demonstrated it's effect on many facets of neuronal physiology and biochemistry. It modifies Na^+/K^+ ATPase in-vitro and in-vivo (Delguado-Escueta and Horan 1980), inactivates sodium channels (MacDonald and Kelly 1993), inhibits neurotransmission (DeLorenzo 1986), blocks L-type calcium channels (Rivet et al 1990), and affects numerous other neuronal biochemical parameters such as chloride permeability, cyclic nucleotide metabolism, and metabolism of GABA, glutamine and glutamate (DeLorenzo 1995). It is unlikely that any one single action is the source of it's anticonvulsant activity, and more probable that this depends on a combination of it's many effects.

Phenytoin has marked activity against partial seizures with or without secondary generalisation. In some developed countries, particularly the USA, it is also drug of choice for primary generalised epilepsies. Adjunctive phenytoin has been found to be of use in patients with multiple seizure types if other drugs fail to control the tonic-clonic component. Intravenous phenytoin is still considered the treatment of choice for status epilepticus (Wilder 1995).

The saturable kinetics and multiple drug interactions exhibited by phenytoin combine to make it a drug that demands careful monitoring during dose titration (Brodie 1990) particularly when it is one of the constituents of polypharmacy. In clinical practice this need for monitoring is a distinct

disadvantage (McKee and Brodie 1994).

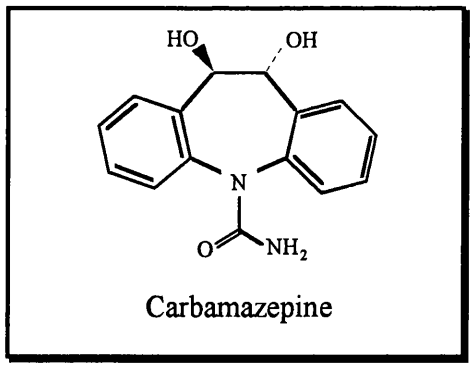
Chronic phenytoin use, even at therapeutic concentrations can cause hirsutism, gum hyperplasia and facial coarsening. Although not life threatening, these cosmetic effects can make the drug unpleasant to use in young women. Vitamin D metabolism can be affected, to the extent of causing hypocalcaemia or osteomalacia in non-ambulant patients (Ashworth and Horn 1977), and interference with folate metabolism can result in a mild macrocytic anaemia of folate deficiency (Jones and Wimbish 1985). There is a recognised risk of fetal abnormality with phenytoin treatment (Hanson and Smith 1975), although on balance, the risks to the fetus of uncontrolled epilepsy would justify phenytoin use in pregnancy in some patients (Brodie and Dichter 1996).

In the UK at least, the rise in popularity of valproate has displaced phenytoin as the treatment of choice for generalised seizures. In other developed countries such as the USA however, phenytoin remains a first-line anticonvulsant drug.

Carbamazepine

Carbamazepine is chemically related to the tricyclic antidepressants, and was first used in trials of antiepileptic activity in 1963 by Theobold and Kunz. It has become one of the first line anticonvulsants in the UK, with particular benefit in localisation-related seizures whether or not there is any secondary

generalisation (Cereghino et al 1974, Troupin et al 1977).



Carbamazepine

Like phenytoin, carbamazepine has been shown to have a wide range of neurochemical and neurophysiological actions (MacDonald 1995a). Although the blockage of sodium channels, which limits sustained repetitive firing, is probably the most important effect, other synaptic effects have been described (MacDonald 1995a). Synaptic effects of carbamazepine include non-competitive NMDA receptor blockage (Lancaster and Davies 1992), adenosine-A1 receptor antagonism (Skerrit et al 1983), enhancement of effect at the $GABA_A$ receptor (MacDonald 1992), and inhibition of uptake of norepinephrine by brain synaptosomes (Purdy et al 1977), although the clinical importance of these is dubious.

In the UK, carbamazepine is considered the drug of choice for any patient with partial seizures, whether or not there is any secondary generalisation. It has documented efficacy against primary generalised seizures, but not against generalised absences, or myoclonic seizures, which may be exacerbated by the introduction of carbamazepine (Sheilds and Saslow 1983).

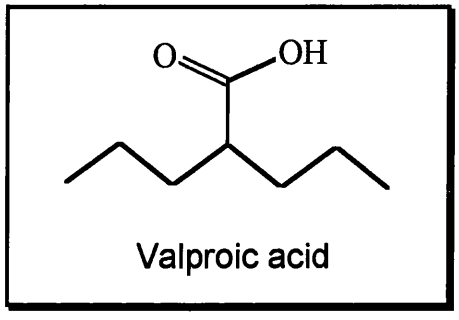
Although it is generally well tolerated, carbamazepine's autoinduction and multiple pharmacokinetic interactions are disadvantageous in terms of general use of the drug (Perucca and Richens 1984, McKee et al 1992), leading to marked inter- and intraindividual variation in response. Rashes of variable

severity occur in up to 15% of patients on carbamazepine, and hyponatraemia can occur in some patients (Gram and Jensen 1989). Haematological monitoring has been shown to be unnecessary, as the risk of agranulocytosis or aplastic anaemia is sufficiently slight (Holmes 1995). Neurological side effects can occur, mainly nausea, headache, dizziness, and diplopia. The incidence and severity of these correlate with the levels of both carbamazepine and it's active metabolite carbamazepine, 10-11-epoxide (CBZ-E) (Patsalos 1985, Gilham et al 1988). The incidence of drowsiness is probably less than with some other first line anticonvulsants (Thompson and Trimble 1982, Andrewes 1986, Gillham et al 1990), although psychomotor testing has shown there to be a discernible negative effect on psychomotor function when carbamazepine is administered to both healthy volunteers (Macphee et al 1986a) and patients with epilepsy (Macphee et al 1986b). Carbamazepine reportedly has positive effects on mood (Loiseau and Duche 1995), although this is not surprising given it's chemical similarity to currently used antidepressant medicines.

Carbamazepine is a highly successful antiepileptic drug, although it does have some limitations. This success has been augmented by the development of the slow release preparation, which leads to an increased tolerability via a decrease in plasma level variability, while not significantly reducing bio-availability (McKee et al 1991).

Sodium valproate

The discovery of the anticonvulsant properties of sodium valproate were rather serendipitous. In 1963, valproic acid was being used as a solvent for new anticonvulsants undergoing testing (Meunier et al 1963), when it was first noted as having antiepileptic effects. Valproate was formally licensed for use in 1974, having been tested in most animal models of epilepsy, unlike it's predecessors (Fariello et al 1995).



Valproic acid

The exact mechanism by which sodium valproate exerts its anticonvulsant effect is unknown. It has several effects on neuronal GABA metabolism, including inhibition of succinic semiadehyde dehydrogenase (SSADH) (Harvey et al 1975), and GAD induction (Loscher et al 1991) which combine to increase whole brain GABA content (Godin et al 1969). The inhibitory effect on depolarisation-induced gamma-hydroxybutyrate release (Vayer et al 1988) may be of some importance, as may blockage of voltage-sensitive sodium channels (MacDonald and Kelly 1993), an increase in Ca^{2+}-dependent K^{+} influx (Francesschetti et al 1986), and a decrease in concentration of excitatory amino acids such as aspartate (Schechter et al 1978).

Valproate's efficacy against generalised tonic clonic seizures (Gram and Bentsen 1984, Shakir et al 1981) and partial seizures (Shakir et al 1981) have been confirmed. Other seizure types, such as myoclonic epilepsy and absence attacks (Sato et al 1982, Gram and Bentsen 1985, Chadwick 1990) are ameliorated by valproate, and the drug is considered treatment of choice

in both conditions (Brodie and Dichter 1996).

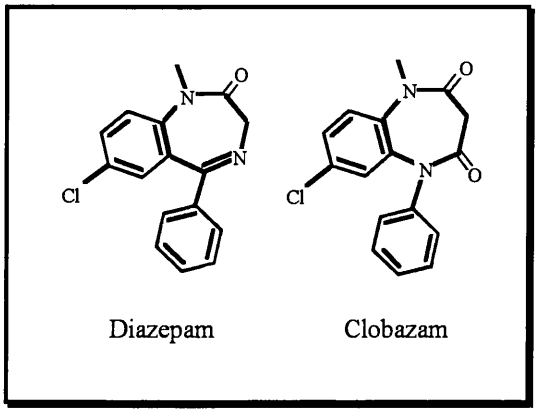
Adverse effects of valproate include tremor, hair loss and weight gain (Gram and Bentsen 1985) the latter two causing problems particularly among young female patients. Dose reduction may partially solve these, but withdrawal of the drug may be necessary in some patients. Rarer, though more serious, are the episodes of hepatitis, hepatic failure, pancreatitis, thrombocytopenia, and coma (Dreifuss 1989) which have been associated with sodium valproate use. The adverse effects, particularly the hepatic ones, are more common in children, but their rarity has ensured that valproate is still considered to be safe. Use of the compound in countries such as the USA is still limited as a result of concerns regarding these adverse effects.

Valproate does have some advantages over other first line agents, including a lack of sedation (Thompson and Trimble 1981, 1982, Gillham et al 1991). Cognitive effects are less severe than with other AEDs, and valproate use has been recommended where psychomotor performance is an important consideration (Gilham et al 1991).

Valproate is not an enzyme inducer, and unlike the other first line AEDs, it does not display autoinduction or saturable pharmacokinetics. Neither efficacy nor toxicity of valproate can be correlated with plasma levels (Chadwick 1985, Brodie and Feely 1988), so serum level monitoring is not necessary in patients on valproate monotherapy. Drug interactions are less troublesome than with enzyme-inducing AEDs, although valproate has some enzyme inhibiting properties (Kapetanovic and Kupferberg 1980, Koch et al 1981). This is of clinical significance when the drug is added to existing

anticonvulsant treatment regimes (Levy and Koch 1982, Leach and Brodie 1995). In summary, valproate is one of the most important anticonvulsants in clinical use, and along with carbamazepine, is one of the most commonly used in the UK.

Benzodiazepines

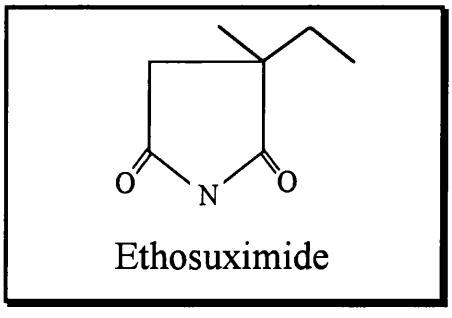


In 1960, Randall and colleagues demonstrated the efficacy of the benzodiazepine compounds in preventing seizure induction by maximal electroshock and pentylenetetrazol. This family of compounds probably exert their anticonvulsant effect on binding with their specific binding site on the γ-subunit of the $GABA_A$ receptor (Ehlert 1986), which results in an increased hyperpolarisation of affected neurones (Twyman et al 1989).

An additional effect on sodium channels has been described, similar to that caused by carbamazepine, phenytoin, and sodium valproate. This blockage occurs at concentrations of benzodiazepine attained during treatment of status epilepticus, and, as it is not blocked by flumazenil (McLean and MacDonald 1988), it would appear to be independent of the effect on $GABA_A$ receptors. The reported changes in calcium channels (MacDonald 1995b) occur at concentrations higher than those attained during treatment, and would appear to have little clinical relevance.

Benzodiazepines still have a place in the immediate, parenteral treatment of status epilepticus (Simon 1985). Although diazepam is the benzodiazepine most commonly used in this situation, the derivatives clobazam (Shorvon 1995) and clonazepam (Shakir et al 1979, Mikkelsen et al 1981) have been shown to have beneficial effects against a wide range of seizure types when given orally as adjunctive long-term treatment. Clobazam is less sedative than the older benzodiazepines (Hindmarch and Gudgeon 1980, Trimble and Robertson 1986) but as with the barbiturates, tolerance (Trimble and Robertson 1986) and withdrawal seizures (Fialip et al 1987, Allen et al 1983) can be a problem. Despite this, clobazam can be useful when given as intermittent adjunctive treatment, as in the treatment of catamenial epilepsy (Feely et al 1982).

Ethosuximide



Ethosuximide

First introduced in 1958, this drug remains a useful compound in paediatric practice in the treatment of absence seizures (Brodie and Dichter 1996). It acts by blocking voltage-dependent calcium conductance in thalamic neurones. Side effects of ethosuximide use are either gastro-intestinal (nausea, vomiting, abdominal pain) or involving the central nervous system (lethargy, dizziness, ataxia).

Ethosuximide does not affect the metabolism of other drugs, but its own

metabolism is affected by enzyme-inducing or inhibiting antiepileptic drugs (Pisani et al 1990). The efficacy and safety of valproate has ensured that ethosuximide has become a second line treatment for this seizure type (Sato et al 1982).

Monotherapy or Polypharmacy?

For many years monotherapy has been regarded as the ideal management strategy for epilepsy. Publication of a series of studies by Shorvon and Reynolds (1977, 1979, Reynolds and Shorvon 1981) led to the view that AED polypharmacy was largely useful only in producing more adverse events. Noone would argue that anticonvulsant treatment should begin with anything other than a trial of monotherapy, as this will suffice for around two thirds of all newly diagnosed patients. For the remainder, however, polypharmacy is a necessary evil, and it falls to us to formulate a rational strategy to deal with those patients who are refractory to first-choice monotherapy. That our established AEDs have limited efficacy is attested to by an audit at one epilepsy clinic which showed that 42% of patients attending were on long-term AED polypharmacy (Schmidt and Gram 1995). In the normal clinical setting, which is best, monotherapy or polytherapy?

AED Monotherapy

In patients with newly-diagnosed epilepsy, phenobarbitone, phenytoin, carbamazepine, and valproate have shown similar efficacy as monotherapy (Verity et al 1995). Newer drugs such as lamotrigine and vigabatrin, as will be discussed, also have proven efficacy as monotherapy. Since one third of our patients will not be well controlled on monotherapy, what can be done to maximise the chances of its success?

To state the obvious, as a first principle every clinician must aim to commence what is, for each patient, the right drug at the right dose. Proper history-taking

and investigation should ensure that patients with primary generalised epilepsy (Covanis et al 1982), juvenile myoclonic epilepsy, or absence seizures (Chadwick 1990) receive initial treatment with valproate monotherapy. Localisation-related seizures would appear to be better controlled by carbamazepine. Careful expert assessment may reveal the presence of a discrete syndrome, such as Lennox Gastaut syndrome, which may indicate that monotherapy is less likely to be adequate to achieve optimal control (Dulac and N'Guyen 1993). The correct dose of the chosen AED should be used: some studies have shown that increasing the dose of current therapy to maximally tolerated levels attains seizure control in almost one third of those patients who were 'refractory' to monotherapy with phenytoin or primidone (Schmidt 1983). Adverse events can be minimised by avoiding over-rapid dose titration or by using controlled release formulations, particularly with carbamazepine (McKee et al 1991, Persson et al 1990), and less convincingly with valproate (Imaizumi et al 1992).

When one monotherapy fails, it need not necessarily follow that polypharmacy is an inevitable requirement. Hakkareinen (1980) looked at the success rate of alternative monotherapy with carbamazepine or phenytoin when the other had failed, and found that one third of the treatment failures were successfully controlled by the alternative drug. In the Veterans' Administration Study (Mattson et al 1985), 46% of those who were unsuccessfully treated with the first monotherapy chosen, responded to an alternative monotherapy. It should be remembered, however, that in this study some were originally treated with phenobarbitone or primidone, neither of which would today be accepted as

reasonable first line monotherapy.

In summary, AED monotherapy will be adequate for a majority of patients with newly diagnosed epilepsy. It is obviously of paramount importance that the correct steps are taken to ensure that the chances of monotherapy succeeding are maximised. Even if the first drug fails at maximally tolerated doses, a second choice may well prove to be effective as monotherapy in a substantial minority of patients.

Polypharmacy

Polypharmacy has long been held to cause an increased incidence of adverse events (Schmidt and Gram 1995), pharmacokinetic interactions (Brodie 1992), and teratogenesis (Nakane et al 1980) while conferring, at best a limited improvement in efficacy (Reynolds and Shorvon 1981). In one study (Beghi et al 1986), treatment with phenytoin or carbamazepine alone produced side effects in 28 and 38% of patients respectively. Combination of the two, however, produced adverse events in around three quarters of all patients. Lammers et al (1995) following a retrospective review, however, were of the opinion that it was *dosage* rather than the *number* of AEDs used which determined the frequency and severity of adverse events.

Despite polypharmacy's poor image, there can be no doubt that for some patients, even when the 'right' drug is given at the right doses, under carefully controlled conditions, that monotherapy may fail and that polypharmacy will be required to gain full seizure control. In the Veterans Administration Study (Mattson et al, 1985), 39% of 'non-responders' were helped by the addition of

a second drug, with 11% becoming seizure free. One trial of adjuvant valproate in cases where carbamazepine had failed as monotherapy (Dean 1988) showed a similar number of patients improved on combination therapy, with 17% becoming seizure free.

There are good reasons why combination of the older agents would not be universally successful. As described in earlier chapters, each of these drugs has a wide range of actions, while their anticonvulsant efficacy will rely, at least in part, on a non-specific blockage of sodium channels. Combination of these widely active drugs will therefore merely increase the degree of sodium channel blockade while producing a host of more complicated biochemical and physiological effects.

Despite the negative aspects of the older drugs, it is still surprising that so few studies have been carried out to detail which AED combinations will provide maximal benefit with minimal adverse events.

With the advent of a new generation of drugs, there are reasons why polypharmacy should be better tolerated and, perhaps, as efficacious. There have been a number of studies investigating the efficacy and tolerability of specific AED combinations.

Specific combinations

These are discussed in the sections dealing with individual drugs. The combination of old and new (lamotrigine/ valproate, carbamazepine/ vigabatrin), and two new drugs (lamotrigine/ vigabatrin, tiagabine/ vigabatrin) have been attempted with some success, although none has been set out in

the recommended double-dummy design required to discover particular benefit (Richens 1995).

The dearth of trials which examine the optimal combinations of AEDs, may itself be testimony to how poorly we understand their basic mechanisms of actions. The further preclinical investigation of the new AEDs will be vital in this respect. It is probable that the more questions we ask of these drugs, the more surprises they will spring on us. Vigabatrin is a case in point: described for years as a GABA-T inhibitor, we will see in later chapters how it has other actions which may explain some of the drug's antiepileptic effect. Remacemide may be a similar case: having been thought to exert its anticonvulsant effect via NMDA antagonism, its significant effects on GABA metabolism will be demonstrated in one of our models. The preclinical investigation of these two compounds proves that the ancillary modes of action of AEDs are not always easy to predict, and that a comprehensive investigation of each AED's in a wide range of models needs to be carried out.

Any patient studies investigation drug combinations will be complicated by the presence of any pharmacokinetic interactions. Although less prone to initiate interactions, the newer AEDs' metabolism and disposition is affected by the enzymic effects exerted by their older counterparts. The positive interaction between valproate and lamotrigine which has been described for example (Ferrie and Panayiotopoulos 1994, Brodie et al - submitted), although perhaps partly a pharmacodynamic interaction, is probably magnified by the inhibition of lamotrigine's metabolism by valproate.

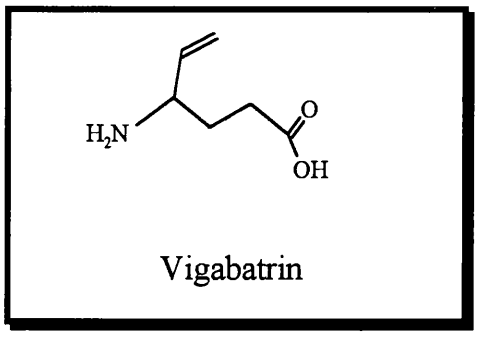
In summary, monotherapy would be the ideal to which everyone would adhere if we had anticonvulsants that were universally efficacious. This is obviously not the case at present. As a result polypharmacy is, for a significant minority of patients, a necessary evil. With a new generation of drugs available with novel anticonvulsant actions, our next challenge is to optimise AED combinations to allow improved efficacy and tolerability for patients who are currently refractory.

The new anticonvulsant drugs

The following chapter deals with the drugs that have been developed in the last fifteen years. With the exception of lamotrigine, they are either awaiting full approval in the UK, or remain licensed only for use as add-on therapy in refractory epilepsy. The development of new AEDs is continuing at an unprecedented rate, and more compounds will certainly be licensed for use in this country in the next few years.

This chapter deals largely with the drugs that are either licensed for use in the UK (vigabatrin, lamotrigine, gabapentin, topiramate), those that may be licensed for use in the near future (oxcarbazepine, tiagabine), those that came close to being licensed (felbamate) and those that have undergone particular study as part of this thesis (remacemide). There are other compounds which are at various stages of development, such as levetiracetam, zonisamide, losigamone, stiripentol and CGP33 101 (Stables et al 1995). They are not dealt with in any great detail in this thesis.

Vigabatrin



Vigabatrin

Vigabatrin (gamma-vinyl GABA) was the herald of a new era in the therapy of epilepsy, being the first 'designer' AED (Leach and Brodie 1995). Binding of vigabatrin to GABA-transaminase leads to an irreversible covalent bonding which inactivates the GABA-T, hence vigabatrin's description as a 'suicide inhibitor' of GABA-T (Leach and Brodie 1995). The increase in whole brain GABA which results from decreased GABA-T activity is thought to lead to an increase in synaptic GABA levels and an augmentation of GABAergic inhibition. Vigabatrin was granted a licence in the UK in 1989.

Mechanisms of action

The S-enantiomer of vigabatrin is a very specific enzyme inhibitor, while the R-enantiomer is completely inactive (Haegele and Schechter 1986). In vitro studies would suggest that the S-form acts only against GABA-T, being essentially inactive against GAD, ornithine transaminase, and aspartate transaminase even at high concentrations in-vitro (John et al 1979, Jung and Palfreyman 1995). In vivo studies have shown that there is a decrease in GAD activity following exposure to high dose vigabatrin, although whether this is secondary to the raised GABA or to the vigabatrin itself is a moot point (Leach et al - submitted, Jung et al 1977, Horton 1989, Jung and Palfreyman 1995). Some studies have shown that concentrations of glutamate, aspartate and

glutamine may be favourably affected in specific areas of the brain such as the hippocampus (Halonen et al 1991), perhaps a sign that vigabatrin may exert its effect via other mechanisms.

Other changes in the GABAergic system have been reported. Some inhibition of GABA release by vigabatrin has been described (Abdul-Ghani et al 1980), although tolerance to this effect has been demonstrated in vitro (Neal and Shah 1990). Some work has suggested that some of the anticonvulsant effect of vigabatrin may come from a direct effect on the $GABA_A$ receptor (Xu et al 1991), although this has not been widely confirmed.

Preclinical testing

Vigabatrin affords good protection against audiogenic seizures in DBA/2 mice (Schechter et al 1977), photically induced seizures in baboons (Meldrum and Horton 1978), and other reflex-induced seizures in other species (Jung and Palfreyman 1995). The duration of absence seizures in Wistar rats is increased by vigabatrin, an effect that is antagonised by diazepam (Marescaux et al, 1985).

Pharmacokinetics and interactions

Being a small, water soluble molecule, vigabatrin is rapidly absorbed after oral administration (Ben Menachem 1995). Peak serum levels occur at around 2 hours after dosing, and the drug is not significantly protein bound (Schechter 1989). Despite the differences in the enantiomers' pharmacodynamic activity, vigabatrin is given as a racemic mixture.

The majority of each dose is excreted unchanged in the urine, the plasma elimination half life being between 5 and 8 hours (Ben Menachem 1995) in normal subjects. As a result of the site and mode of action, however, the pharmacological effect of vigabatrin is much longer than it's pharmacokinetics would suggest, levels of brain GABA remaining high up to 120 hours post dose (Schechter et al 1977). Clinical effects in man last up to 48 hours post-dose (Ben Menachem 1990). Dose-related increases in CSF GABA levels have been observed following vigabatrin administration (BenMenachem et al, 1989), which have been postulated to be related to anticonvulsant efficacy (Riekkinen et al 1989).

As would be expected with a drug that is excreted entirely unchanged in the urine, no clinically important interactions with other anticonvulsants have been described. Vigabatrin has been shown to decrease serum phenytoin via an unknown mechanism by a mean of 20% (Rimmer and Richens 1989), but in only one (open!) study has this been thought to compromise seizure control (Browne et al 1987).

Efficacy

Many studies have confirmed the efficacy of vigabatrin as add-on therapy for refractory epilepsy in adults (Browne et al 1987, Gram et al 1985, Remy et al 1986, Rimmer and Richens 1984, Tassinari et al 1987, Reynolds et al 1991, Browne et al 1991, Loiseau et al 1986) and children (Herranz et al 1991, Luna et al 1989). On average, 50% of patients had their seizure frequency at least halved, and of those who had no change in frequency, the seizure severity

was often found to be reduced (Sander et al 1990, Tassinari et al 1987). When used as add-on therapy, greater efficacy is seen in patients with partial seizures (Michelucci et al 1989). The reported effect on generalised seizures is variable (Browne et al 1987).

A meta-analysis of the European placebo-controlled studies (Mumford and Dam 1989) looked at the 398 patients who had been enrolled in studies in the early and mid-1980s. This showed a reduction in seizure frequency associated with vigabatrin use, confirming that this was most marked in those patients with partial seizures whether or not there was any secondary generalisation.

On controlled comparative testing against steroids, vigabatrin has been shown to be the superior treatment for infantile spasms (Chiron et al 1991), and some authorities have suggested that it has become treatment of choice for West syndrome (Appleton 1995)

Withdrawal due to poor tolerability of vigabatrin is much the same as for other treatments, i.e. 5 - 15% (Browne et al 1987), and tolerance is not thought to be a feature of it's long term use. Long term follow up shows that even up to 6 years after commencing treatment there is no evidence of tolerance (Tartara et al, 1992). The efficacy of vigabatrin may not be improved by dose increases beyond 2 grams per day (McKee et al, 1993), possibly as a result of the GAD inhibition that occurs.

Specific combinations of drugs are not commonly tested in the field of antiepileptic medication. One trial has been published which proves vigabatrin's role as a good add-on drug when combined with carbamazepine

(Murri and Ludice 1995) in 40 patients with refractory localisation-related epilepsy. There was a significant reduction in mean seizure frequency, and 17.5% of patients became seizure free on the combination. The authors conclude that vigabatrin should be an early choice of drug for add-on therapy.

Monotherapy trials

One monotherapy trial has so far been published, comparing vigabatrin monotherapy with carbamazepine monotherapy (Kailviainen et al 1995). A total of 100 patients were followed for up to 12 months. Sixty percent of both treatment groups completed one years treatment being rendered either seizure-free or having an acceptably low seizure frequency. Vigabatrin was better tolerated, no patients requiring withdrawal because of side effects (against 24% of the carbamazepine group). Significantly more patients in the carbamazepine-treated group were rendered seizure-free (52% on carbamazepine against 32% on vigabatrin), with fewer patients on the established drug requiring withdrawal due to lack of efficacy (6% on carbamazepine against 26% on vigabatrin).

Toxicity

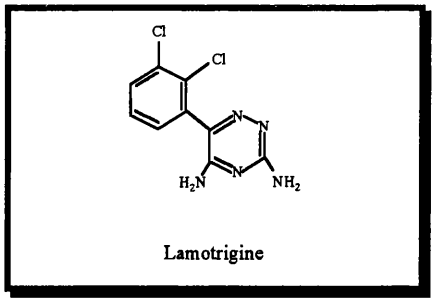
A review of over 2000 patients on vigabatrin (Grant and Heel 1991) gave the incidence of drowsiness at around 10%, with some dizziness, headache, diplopia, ataxia and vertigo reported in around 2%. Psychiatric side effects such as anxiety, depression, and aggression are well recognised: the precipitation of psychosis at high doses or following sudden withdrawal of the

drug should lead to cautious use of vigabatrin in those with a history of psychiatric illness (Brodie and McKee 1990, Sander et al 1991) This association of vigabatrin with psychosis is likely to be more than an association between the drug and so-called forced normalisation. Maintenance at low dose may avoid psychiatric problems while still providing some benefit (Brodie and McKee 1990).

Neuronal cytoplasmic vacuoulation, though visible in rodents and dogs after chronic dosing (Hammond and Wilder, 1985; Butler et al, 1989), has not been seen in human or even primate brain despite extensive searching (Cannon et al 1991).

The association of high dose vigabatrin and rapid titration of vigabatrin dose with psychosis has probably been damaging for this drug's prospects. The improved tolerability that more cautious use of the drug evokes, however, has allowed its use to steadily increase over the last few years, and it is likely that the drug will be granted a license for use in the USA in the near future. Results from other monotherapy trials are awaited to allow a review of the drug's position in treatment of epilepsy in the twenty first century.

Lamotrigine



Lamotrigine

Folic acid was shown to have proconvulsant properties in the mid-sixties, and anticonvulsant drug development at the Wellcome Institute concentrated on modifying folate antagonists in order to uncover an anticonvulsant compound. Lamotrigine (3,5-diamino-6-[2,3-dichlorophenyll]-1,2,4-triazine) was noted to be both a mild folate antagonist *and* an anticonvulsant, although it is now known that these properties are not linked (Rogawski and Porter 1990).

Mode of Action

Lamotrigine is a phenyltriazine derivative, and is chemically and functionally unrelated to other antiepileptic drugs. The development of burst firing is inhibited by lamotrigine in a manner similar to that of phenytoin and carbamazepine (Macdonald and Kelly, 1993).

At an early stage of investigation (Leach et al 1986), it was found that lamotrigine blocked veratridine rather than potassium-evoked release of endogenous amino acids implying that the anticonvulsant effect arose at least in part from a blockage of voltage-sensitive sodium channels.

Lamotrigine preferentially inhibits release of glutamate, having a lower ED50 for this than for inhibition of GABA release after electrophysiological stimulation (Leach MJ et al 1995). The blockage of sustained repetitive firing by lamotrigine is thought to be a result of the frequency and voltage-

dependency of sodium channel inactivation (Cheung et al 1992, Lees and Leach 1993). This blockage occurs when the channel is at the slow inactivated state (Xie et al, 1995), and this selectivity may account for the drugs tolerability (Brodie and Dichter 1996).

Recent studies have shown that lamotrigine has a mild calcium channel blocking effect: the narrow dose range at which this occurs, however, suggests that this is probably of little clinical import (Lees and Leach 1993).

Lamotrigine does not bind to adenosine, $GABA_B$ or opioid receptors (Leach MJ et al 1995), although there is some weak binding to the 5-HT3 receptor which is of dubious functional significance. Lamotrigine has no effect on either $GABA_A$ or NMDA receptors (Leach MJ et al 1995).

Pharmacokinetics and Interactions

Oral administration leads to rapid and near complete absorption of lamotrigine. The elimination half life is around 29 hours (Cohen et al 1987), with metabolism largely by hepatic glucuronidation. The most common metabolite (70% of the dose) is the N-2 glucuronide conjugate (Magdalou et al 1992). Interestingly, patients with Gilbert's disease demonstrate a longer lamotrigine half life due to a decrease in the activity of diphosphate glutamyl transferase (Posner et al 1989).

Lamotrigine does not induce or inhibit hepatic enzymes. Consequently, it has no influence on the metabolism of other lipid-soluble drugs, such as the oral contraceptive pill and warfarin (Leach and Brodie 1995). There have been reports of symptoms of neurotoxicity (headache, nausea, dizziness, diplopia,

ataxia) in patients when lamotrigine is added to a stable carbamazepine regime (Warner 1992). This has since been shown to be a pharmacodynamic rather than a pharmacokinetic interaction (Stolarek et al 1994, Pisani et al 1994).

The half life of lamotrigine is more than doubled by concomitant valproate to around 59 hours (Yuen et al 1992), while enzyme-inducing anticonvulsants such as carbamazepine and phenytoin have the opposite effect, reducing it to around 12 hours (Binnie et al 1989).

Preclinical testing

Lamotrigine is active against the tonic phase of both PTZ- and MES-induced seizures (Yuen 1991), blocks the development and expression of amygdaloid kindled seizures (Miller et al 1986), and reduces electrically-evoked afterdischarge duration in the rat, dog, and marmoset (Wheatley and Miller, 1989). It is, however, ineffective against both threshold and clonic seizures induced by PTZ (Rogawski and Porter 1990). The reduction in glutamate release is likely to account for the long-term neuroprotective actions of lamotrigine shown in animal models whether given before or after the onset of ischaemia (Smith et al 1995, Shuaib et al 1995).

Efficacy

Eleven double-blind placebo-controlled studies have been published (Jawad et al 1989, Sander 1990; Loiseau et al 1990; Binnie et al 1989; Risner et al 1990, Dren et al 1991, Schapel et al 1993, Smith et al 1993, Matsuo et al

1993, Messenheimer et al 1994, Schachter et al 1995), showing success as add-on treatment of partial seizures with or without secondary generalisation. Only the most relevant will be discussed here.

Messenheimer and colleagues (1994) examined 98 patients with localisation-related epilepsy, as part of a double blind placebo controlled crossover trial, with patients titrated to a maximum tolerated dose not greater than 400mg/day. 44% of the subjects experienced a reduction in seizure frequency of at least 25%, with 20% having their seizures frequency at least halved.

A double-blind placebo-controlled study comparing the efficacy of lamotrigine at 300mg/day with 500mg/day (Matuso et al 1993) in 216 patients with refractory partial seizures showed the higher dose to cause a significantly greater reduction in seizure frequency compared to both the lower dose and placebo. 300mg/day was not significantly better than placebo.

Schapel et al (1993) during a relatively short treatment period showed a significant reduction in partial seizure numbers and total seizure numbers with lamotrigine titrated up to a maximum tolerated dose not greater than 300mg/day. There was a trend towards reduction in secondary generalised seizures, although this did not reach statistical significance. One of the largest studies (Schachter et al 1995) involved 446 patients in a six-month treatment period, showing a beneficial effect of lamotrigine when used as add-on therapy for refractory partial seizures. Alongside its anticonvulsant effect, lamotrigine has been shown in one study to have a positive effect on some measures of happiness and mastery (Smith et al 1993).

Lamotrigine has been said to work particularly in combination with some other

anticonvulsant drugs, particularly sodium valproate (Ferrie and Panayiotopoulos 1994) and vigabatrin (Robinson et al 1993, Stolarek et al 1994). Schapel and colleagues assessed the vigabatrin\ lamotrigine combination in a study of 42 patients. In this refractory population 40% had a reduction in seizure frequency of at least 80% compared to baseline, and 69% had their seizure frequency at least halved while on both new drugs. Addition of one of the new agents did not cause a significant reduction in mean seizure frequency, and no one drug was more efficacious than the other. Only on addition of the second drug was there a significant reduction in seizure frequency, which persisted despite withdrawal, or at least reduction in the baseline AED therapy. Perhaps significantly, 30 of the patients were also receiving valproate in the third phase, which allowed them to tolerate a higher plasma lamotrigine concentration.

Brodie et al (paper submitted) as part of a withdrawal to monotherapy study in 347 patients, showed a better response to add-on lamotrigine in those receiving valproate (n=117) compared to those on carbamazepine or phenytoin. This improvement occurred irrespective of seizure type and patients on valproate had similar plasma lamotrigine levels. On withdrawal of valproate, despite an associated rise in lamotrigine dose to raise the lamotrigine levels above those in the add-on phase, valproate-treated patients, unlike those on other baseline therapies had a loss in seizure control, suggesting that the benefits of combined treatment were greater.

Experience in some units suggests that lamotrigine also has great success in primary generalised epilepsy (Stewart et al 1992, Richens 1994). It has been

suggested that lamotrigine may have efficacy in the Lennox-Gastaut syndrome (Timmings and Richens 1992), juvenile myoclonic epilepsy (Timmings and Richens 1993), and Rett Syndrome (Uldall et al 1993). Any suggested benefit in neuroprotection during cerebral infarction or Parkinson's disease (Zipp et al 1993), has not yet been substantiated by controlled trials.

Monotherapy trials

Brodie et al (1995) as part of a large double-blind trial comparing efficacy of lamotrigine monotherapy with that of carbamazepine monotherapy, followed 260 patients with newly diagnosed localisation related epilepsy over a 48-week treatment period. Doses of both treatments were controlled by an unblinded observer on the basis of serum levels, in order to maintain serum levels within an arbitrary (for lamotrigine) target range. During the treatment period, once doses were stabilised, lamotrigine showed similar efficacy to that of carbamazepine when measured by time to first seizure. During the last six months, almost identical numbers (38% on carbamazepine, 39% on lamotrigine) remained seizure free.

On comparison of tolerability, however, lamotrigine was clearly preferable. Fewer patients on lamotrigine withdrew from the study because of adverse events (27% of those on carbamazepine, 15% of those on lamotrigine). It was mainly as a result of this and other trials that lamotrigine was given a licence for use as monotherapy in the UK and elsewhere in 1995. A similar comparative trial with phenytoin has been carried out which shows similar benefits of lamotrigine treatment compared to phenytoin monotherapy in a

similar patient group (Steiner and Yuen 1994).

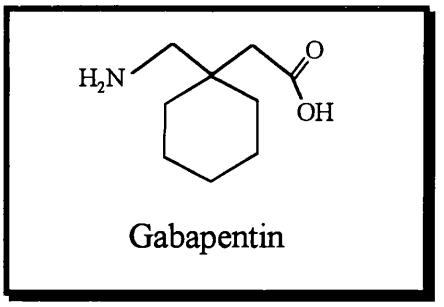
Toxicity

By November 1992, more than 5800 patient years experience had been gained with lamotrigine, mostly as add-on therapy (Richens 1994). Skin rash occurs in around 5% of those involved in clinical trials with lamotrigine (Richens 1994), but requires withdrawal from trial in only 2% of patients (Messenheimer et al 1994) a figure substantially lower than the incidence during carbamazepine treatment. Rechallenge of patients at lower starting dose and slower titration of lamotrigine dose can be successful in avoiding reemergence of the rash (Tavenor et al 1995).

As described above, there is a well-defined pharmacokinetic effect of sodium valproate on the kinetics of lamotrigine which increases the risk of onset of adverse events. This interaction necessitates a reduced starting dose and reduced lamotrigine titration rate in patients on regimes containing valproate (Leach and Brodie 1995).

Mild central nervous system events, such as dizziness, ataxia, drowsiness, headache, and diplopia, occur with lamotrigine use in a dose-dependent manner, although the side effect profile compares favourably with that of the established anticonvulsants (Richens 1994).

GABAPENTIN



Gabapentin

Gabapentin [1-(aminomethyl) cyclohenanacetic acid] is an anticonvulsant drug which was given a licence for use in the UK as add-on therapy in 1993. It was developed in an attempt to exploit a presumed direct GABAmimetic effect (Ojemann et al 1988). GABA itself does not cross the blood brain barrier when given systemically, and work was carried out in order to manipulate the structure of GABA to allow a direct GABA agonist to do this with ease. Further work has demonstrated gabapentin's lack of binding to GABA receptors (Bartoszyk et al 1986).

Mode of Action

The full effects of gabapentin on specific receptors has not yet been clearly defined, although it has been shown that there is no direct action on $GABA_A$ or $GABA_B$ receptors, and no effect on benzodiazepine receptors (Schmidt 1989). There may be a specific, previously undescribed receptor for gabapentin, similar to one binding 3-isobutyl GABA (Taylor et al 1993, Hill et al 1993).

Enhancement of GABA release may be important in the actions of gabapentin (Honmou et al 1995). At therapeutic concentrations, gabapentin increases GABA release from neostriatal slices in-vitro, an effect that is blocked by $GABA_A$ receptor antagonists (Gotz et al 1993). Gabapentin does not alter GABA uptake into neurones or astrocytes (Schmidt 1989).

The activity of GAD is said to be increased by gabapentin (Loscher et al 1991, Taylor et al 1992), and other enzymes of importance in the GABA shunt have been shown to be affected in the presence of gabapentin, including a minor inhibition of GABA-T, and more significant inhibition of glutamate dehydrogenase, and branched chain amino acid transferase (Goldlust et al 1995). It was felt that the reduction in glutamate synthesis may be more important mechanism of action than an increase in GABA. The effects of gabapentin treatment on glutamate levels has yet to be assessed by this method.

CSF GABA levels have previously been shown to be unchanged by gabapentin after single dose (Ben Menachem et al 1992), or three months treatment (Ben Menachem et al 1995). NMR spectroscopy in man, however, has suggested that an elevation of GABA occurs on treatment with gabapentin which is more marked at higher doses (>3gm/day) than with 'standard' doses (1.2-2.4gm/day) (Petroff et al 1996).

The peak anticonvulsant effect of gabapentin lags some two hours behind peak serum levels (Welty et al 1993), supporting the theory that enzyme inhibition may be the principle mode of action.

Thurlow et al (1993) have shown binding to a site proposed to be a cellular L-amino acid transporter, suggesting that gabapentin could affect transport of other neurotransmitter amino acids in vivo.

Pharmacokinetics and Interactions

Oral dosing of gabapentin gives rapid absorption, and the drug has a

bioavailability of 60%. Maximum levels occur 2-3 hours after administration and the half life is 5-7 hours (Vollmer et al 1986). There is no significant protein binding, and the drug is excreted unchanged in the urine (Vollmer et al 1986, Stewart et al 1993) with clearance rates equivalent to creatinine clearance. This rapid clearance currently would suggest that three times daily dosing is required with gabapentin, although some clinicians suspect that twice daily dosing may be equally effective.

Unsurprisingly, gabapentin's pharmacokinetic characteristics are not changed with chronic dosing, and the lack of drug interactions with other AEDs (Tyndel 1994, Radulovic et al 1994, Hooper et al 1991) and the oral contraceptive pill (Eldon 1993) has been widely reported. Stewart and colleagues (1993) have demonstrated a disproportionately small rise in serum levels on increasing gabapentin dosage, possibly because of a saturable intestinal L-amino acid transport mechanism.

Preclinical testing

Work in rodents (Bartosyk et al 1986) has shown gabapentin to be effective against a wide variety of electrical, physical and chemical stimuli. Anticonvulsant effects were apparent against NMDA induced seizures, but not those produced by kainate or quisqualate (Bartosyk et al 1986). Gabapentin has been shown to have an experimental anticonvulsant profile similar to that of sodium valproate (Rogawski and Porter, 1990): the drug is effective against tonic seizures induced by a variety of chemoconvulsants including PTZ, bicuculline, picrotoxin, and strychnine (Foot and Wallace 1991), and is also

active in the MES test in rats (Rogawski and Porter, 1990) and against reflex seizures in DBA/2 mice, Mongolian gerbils (Foot and Wallace 1991), and genetically epilepsy-prone rats (Foot and Wallace 1991). It has weak activity in the photosensitive *Papio papio* baboon and was without effect in the kindling model (Foot and Wallace, 1991).

Efficacy

A small double-blind cross-over study (Crawford et al 1987) was the first published work to prove the efficacy of gabapentin, comparing an eight week period on three different doses (300, 600, or 900mg/day) to a similar length of time on baseline observation. At 900mg/day, seizure frequency was significantly reduced, most striking was the reduction in secondary generalised seizures. No changes in psychometric testing were apparent at these doses (Crawford et al 1987).

A later placebo-controlled double-blind study randomised 43 patients to receive placebo or one of two doses of gabapentin (Sivenius et al 1991). After three months, 900mg/day gabapentin was found to be ineffective, while those patients on gabapentin 1200mg/day experienced a significant reduction (57%) in frequency of both tonic-clonic and partial seizures. A 4-year follow-up at the same centre (Sivenius et al 1994) showed that 25% of those remaining on the drug after 4 years had a consistent reduction in seizure frequency.

A later, larger double-blind parallel-group study (UK Gabapentin Study Group 1990) randomised 127 patients to either 1200mg daily of gabapentin or placebo. Compared to placebo, more gabapentin-treated patients (25.0%

against 9.8%) experienced a reduction of >50% in seizure frequency, the mean reduction in seizure frequency after the 12-week treatment phase being 29.2% on gabapentin and 12.5% on placebo.

Anhut et al (1994) used similar doses in their double-blind placebo-controlled study of 272 refractory patients. Response rate and percentage change in seizure frequency were significantly better with gabapentin treatment of either 900 or 1200mg/day. The incidence of adverse events, at first sight seems high in those on gabapentin, 69% and 64% of patients in the low and high-dose groups respectively reporting adverse events. 52% of patients in the placebo-treated group also experienced significant events however.

A large study using higher doses has been carried out. The US Gabapentin Study Group (1993) had 306 patients in four parallel groups on placebo or gabapentin at 600, 1200, or 1800mg/day. A significantly higher number of responders were seen in the 1200 and 1800mg/day groups. Mild to moderate adverse events were reported in 88% of patients on active treatment, compared to 72% of those on placebo.

A pooling of data from double-blind trials (McLean 1995) confirmed gabapentin's efficacy: the 'response rate' (>50% reduction seizure frequency) was 26% of patients suffering from partial seizures, and 54% of those with generalised seizures. The effect on myoclonic and absence seizures does not appear promising (Stables et al 1995) and in our clinical experience, the drug may even exacerbate or precipitate these seizure types, especially at high dose, and in combination with carbamazepine.

Handforth and Treiman (1994) were not alone in suggesting that the drug

could be used at much higher doses to boost efficacy even further, given how well the drug was tolerated at doses used in clinical trials.

Monotherapy Trials

Ojemann et al (1992), as part of an open label trial of gabapentin, successfully converted 5 patients from 9 potential candidates on to gabapentin monotherapy. A prospective short-term double-blinded in-patient study of around 80 patients (Bergey et al 1995) showed that 3600mg/day was better tolerated than 300mg/day when used as monotherapy.

Further, more definitive evidence of the efficacy of gabapentin as monotherapy is awaited. Several trials are underway, and the results should be available shortly.

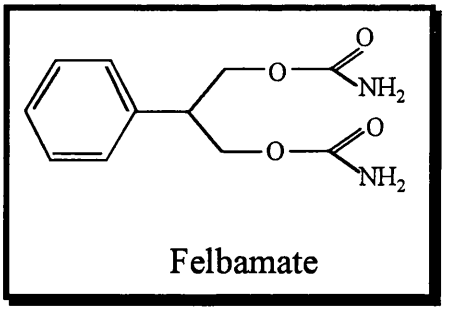
Safety and toxicity

Gabapentin is a very well tolerated drug, and any drug-related problems are usually of early onset, easing after around two weeks. These adverse events are relatively rare, but usually manifest themselves as mild CNS toxicity (Browne 1993), although few studies have been done to assess the psychomotor and cognitive effects of the drug. Gabapentin is not yet licensed for use during pregnancy, and any experience of this has been gained on an ad-hoc basis.

In the report by the UK study group (1990), 62% of those receiving gabapentin reported side effects, the most common being somnolence (14.8%), fatigue (13.1%), dizziness (6.6%) and weight gain (4.9%). In the

placebo group, however, 41% reported side effects including headache (9.1%) followed by dizziness and somnolence (both 4.5%). Of the 11 patients who withdrew due to side effects, seven were on gabapentin

Felbamate



Felbamate

Felbamate is chemically unrelated to the established anticonvulsants, and was approved for use in the USA as add-on and monotherapy in adults with partial seizures alone or with secondary generalisation. The drug was first marketed there in December 1992 (Brodie and Pellock 1995), and subsequently approval was given for use in children with partial or generalised seizures associated with Lennox-Gastaut syndrome. The UK licensing authorities, with a wisdom commendable in hindsight, refused to approve the use of this drug around that time, requesting more proof of efficacy and safety. Later developments would prove their reservations to be well founded.

Mechanisms of action

The mechanisms of action of felbamate are not completely understood, but at therapeutically relevant concentrations it has been shown to reduce voltage dependent sodium currents in a use-dependent manner (analogous to phenytoin and carbamazepine) (White et al 1992), enhance GABAergic inhibition, block the NMDA receptor site (Rho et al 1994), probably with significant glycine antagonism (McCabe et al 1993). Each of these mechanisms could be wholly or partly responsible for felbamate's antiepileptic effect. Felbamate is effective against animal seizure models, including MES- and PTZ-induced seizures. There was a wide separation between therapeutic

and toxic doses.

Pharmacokinetics and interactions

Felbamate is well absorbed orally and is about 22-25% bound to plasma proteins (Perhach and Shumaker 1995). Its plasma half-life is approximately 20 hours, indicating it could be given twice daily, although three to four divided doses are recommended by the manufacturer (Dichter and Brodie 1996). Felbamate undergoes hydroxylation by the liver, although around half of each dose is excreted unchanged in the urine (Shumaker 1990).

There are significant mutual drug interactions between felbamate and the other established AEDs. Felbamate increases phenytoin levels and can precipitate phenytoin toxicity when added to a previously stable regimen (Graves et al 1989). Concomitant felbamate decreases serum carbamazepine levels while increasing levels of carbamazepine-10,11-epoxide (Wagner et al 1993) theoretically producing toxicity at a lower serum carbamazepine levels than would otherwise be expected. Felbamate also increases valproate levels, although inconsistently (Wagner et al 1991). Thus, if felbamate is started in a patient taking any of the established AEDs, concomitant reduction in their dose (by 20-33%) is indicated to prevent toxicity (Dichter and Brodie 1996). Phenytoin and carbamazepine (but not valproate) increase felbamate clearance (Dichter and Brodie 1996).

Efficacy

Three double-blind, placebo-controlled, add-on studies demonstrated

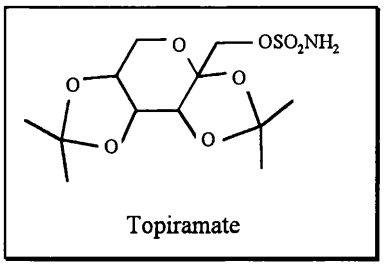
felbamate's efficacy in reducing intractable focal seizures in adults compared to placebo (Theodore et al 1991, Leppik et al 1991, Bourgeois et al 1993). In two innovative trial designs, utilising monotherapy in patients in whom conventional therapy was withdrawn (Sachdeo et al 1992), and in patients being evaluated for epilepsy surgery whose concomitant AEDs were being discontinued (Faught et al 1993), felbamate was shown to be better than placebo or low-dose valproate in preventing the recurrence of seizures. In children with the Lennox-Gastaut syndrome (Felbamate Study Group 1993), felbamate was demonstrated to be superior to placebo for reducing total seizure frequency and reducing atonic seizures.

Within controlled clinical studies, felbamate was considered to be a well tolerated compound, many of the documented side-effects being attributed to the interaction of felbamate with concomitant AEDs. Felbamate use was associated with nausea, decreased appetite, insomnia, agitation and headache (Liporace et al 1994, Bebin et al 1995). Post-marketing, however, these side-effects appeared to be more prominent and resulted in a significant number of patients discontinuing the medication (Wolff et al 1994). Within a year, after around 100,000 patients had been exposed to felbamate, two very serious problems arose. By 1995, aplastic anaemia had developed in 32 patients, and hepatic failure in 19 patients (Brodie and Pellock 1995). It has been reported that five of those with hepatotoxicity, and ten of those with bone marrow suppression have died (Brodie and Pellock 1995).

Felbamate use in the UK is rare, and on a named patient basis only. In the USA, however, the FDA have restricted use to those patients refractory to all

other medications and in whom the risk-benefit relationship is favourable. Weekly or bi-weekly blood counts and liver function tests must be performed, although it is not known whether early detection of either of these idiosyncratic reactions will prevent the most serious outcomes.

Topiramate



Topiramate

Topiramate [2,3:4,5-bis-O-(1-methylethylidene)B-D-fructo-pyranose sulfamate] is chemically unrelated to other AEDs, deriving as it does from D-fructose, and containing a sulfamate functionality (Shank et al 1994). In November 1995, topiramate received its UK license for use as add-on therapy for refractory epilepsy.

Mode of Action

The precise mechanisms by which topiramate exerts its anticonvulsant effect are as yet unknown. It is likely that, like the established anticonvulsants, topiramate has several effects on neuronal physiology and biochemistry, and that the anticonvulsant activity depends on a combination of these effects.

Like the established AEDs, topiramate decreases sodium channel conductance (Taylor 1993b), an action which occurs at a wide range of doses, and which prevents sustained repetitive firing (Coulter et al 1993). As with barbiturates and the benzodiazepines, topiramate's efficacy may depend to a degree on the potentiation of GABA's action on chloride channel conductance (Brown et al 1993): topiramate increases the frequency rather than the duration of chloride channel opening in response to GABA (Twyman et al - In Press), an action which is not blocked by flumazenil (White et al 1995).

A modest block of AMPA and kainate receptors has been described (Coulter et al 1993a, Stables et al 1995), although the relative importance of this

blockade is as yet unknown. The drug is known to have some carbonic anhydrase inhibitory activity (Shanks et al 1994), although this is mild across several species, and is felt to have little bearing on its anticonvulsant action.

Pharmacokinetics and interactions

In man, topiramate has 80% bioavailability, which is unchanged with concomitant food ingestion. It is weakly (15%) protein bound, and is largely eliminated unchanged by the kidney. In non-enzyme induced patients, around 20% is metabolised, and when used as monotherapy, the drug has a half life of around 20-30 hours. Pharmacokinetics are linear, the plasma level increasing in proportion to the dose. Of the fraction that is metabolised by the liver, there are six different metabolites formed: none have any important anticonvulsant actions. In patients with renal or hepatic impairment, and in whom elimination or metabolism is decreased, lesser doses of topiramate may be required to avoid dose-dependent side effects.

The established enzyme-inducing AEDs increase the clearance of topiramate. Phenytoin increases the *total* clearance by a factor of 2 or 3 (Willensky et al 1989), while carbamazepine increases only the non-renal clearance (Floren et al 1989). Concomitant valproate (Floren et al 1989), like phenobarbitone, does not significantly affect topiramate clearance.

Topiramate exerts no effects on the metabolism of the established AEDs other than phenytoin. In this case topiramate reduces total clearance of phenytoin to a variable degree (Floren et al 1989), occasionally by as much as 15%, leading to occasional rises in phenytoin steady state concentrations of as

much as 25%. Notwithstanding this, it is reported that there is usually no change in phenytoin dose required in patients commencing topiramate.

In relation to other drugs, it is known that clearance of both digoxin and oestrogen are increased by topiramate. This may have important connotations for the drug's usage in old and younger patient groups respectively.

Preclinical testing

In both rats and mice, topiramate has been shown to be efficacious against MES one hour after dosing, with a potency similar to that of phenytoin or carbamazepine (Shank et al 1994). In the case of treated rats, the anticonvulsant effect was still evident 24 hours after dosing. Potency in rats was 2-5 times greater than in mice (Shank et al 1994), the converse of the situation with phenytoin. In animal models, the D-isoform is between 2 and 5 times more potent than the L-isoform.

In mice, topiramate had only a weak effect against those seizures provoked by PTZ (Shank et al 1994), but had no effect against those induced by picrotoxin or bicuculline even 4 hours after dosing with topiramate at 800mg/Kg. Like phenytoin, topiramate did not block PTZ, or DMCM-induced seizures up to 6 hours after dosing.

Efficacy

Double-blind trials of anticonvulsant efficacy in patients with epilepsy have been carried out, although fully published information is scanty and most data are only available in abstract form. At 400mg daily in 46 patients (Martinez-

Lage et al 1995), 22% of the topiramate-treated group had their seizure frequency reduced by at least 75%, compared to 4% of those in the placebo-treated group. The commonly used 'responder rate' (>50% reduction in seizure frequency) was 35% and 8% for topiramate and placebo groups respectively.

In another placebo-controlled study involving 60 patients (Tassinari et al 1995), topiramate at least halved the seizure frequency in 47% of topiramate treated patients at 600mg/day (10% placebo). 23% of those on active treatment had their seizures reduced by 75% compared to only 3% of the placebo-treated group. There were no serious adverse events reported during either of these trials.

In another more complex study involving 56 patients (Ben Menachem et al 1995b), patients were given either 800mg/day or the maximum tolerated dose, whichever was the greater. None of the placebo treated group had their seizure frequency reduced by 50% or more. In contrast, 43% of the actively treated group had their seizure frequency at least halved, with 36% having seizure frequency reduced by at least 75%.

Assessment of higher doses has been attempted (Privitera et al 1995). Comparison of placebo treatment with topiramate at higher doses showed that the 'responder rate' was 44%, 40%, and 35% at 600, 800, and 1000mg daily respectively compared to only 9% of the placebo treated group.

So far 70 patients have been reported as having been treated with topiramate for over five years with no loss of efficacy (Stables et al 1995).

Monotherapy Trials

A comparison of low (100mg/day) and high (1000mg/day) topiramate when given as monotherapy in newly diagnosed patients (Sachdeo et al 1995) showed more patients receiving the higher dose remained seizure-free at the conclusion of the 112-day study period. More information is required before these results can be easily interpreted.

Tolerability

Many of the side effects experienced during the clinical trials are said to be a consequence of the rapid titration schedule. Certainly the generally accepted titration rates are less than those used during the trial programme, although whether this relaxation will ease the side effect profile is still to become apparent.

The most commonly described adverse events (Ben-Menachem et al 1995b) involved the central nervous system, including ataxia, dizziness, poor concentration, asthenia, paraesthesiae and weight loss. Meta-analysis of all clinical trials has confirmed that nephrolithiasis occurs more commonly during treatment with topiramate (Wasserstein et al 1995a), probably due to a treatment-related decrease in urinary citrate excretion (Wasserstein et al 1995b). Most renal calculi were passed spontaneously and asymptomatically.

Teratogenesis has been demonstrated at a wide range of doses in mice (BenMenachem et al 1995b), but only at the highest doses used in rats (500mg/kg/day). No teratogenic effect was seen in rabbits at any dose tested (BenMenachem et al 1995b).**Oxcarbazepine**

Oxcarbazepine (oxcarbazepine) is the 10-keto analogue of carbamazepine whose modifications ensure that it has a different metabolic profile from its ancestor. oxcarbazepine is essentially a pro-drug (Dichter and Brodie 1996), which is rapidly and completely reduced in the liver to the active moiety,

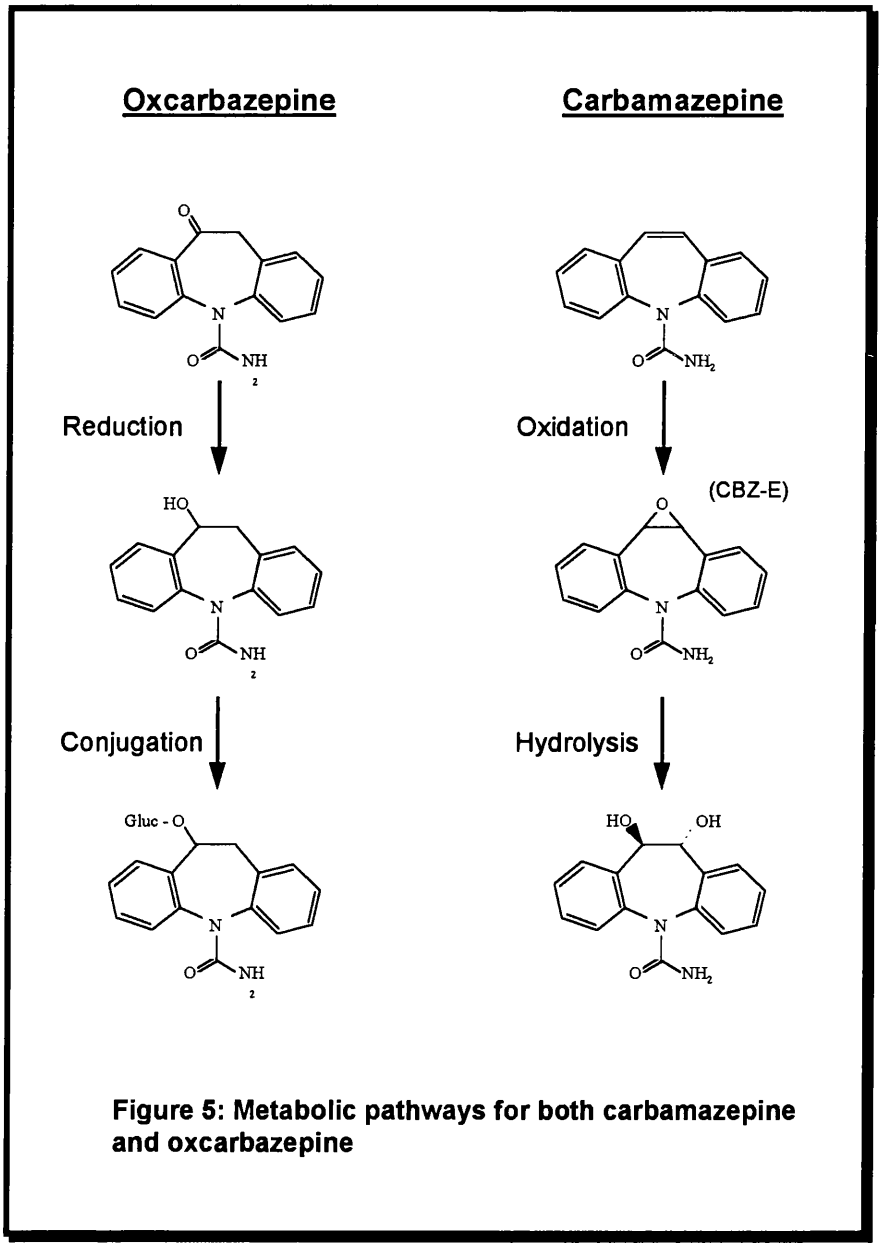


Figure 5: Metabolic pathways for both carbamazepine and oxcarbazepine

10,11-dihydro-10-hydroxycarbamazepine (OHCBZ) (Figure 6). The main advantage of oxcarbazepine arises from avoidance of formation of carbamazepine 10,11 epoxide (CBZ-E) (Patsalos and Sander 1994) during its

metabolism. As described earlier (See *Established Antiepileptic Drugs - Carbamazepine*), CBZ-E accounts for many of the adverse events experienced during carbamazepine treatment (Patsalos et al 1985, Gillham et al 1988).

Mode of action

There is little specific information available on the mechanism of action of oxcarbazepine, as it has always been thought to be closely related to that of carbamazepine. Certainly the effect of both compounds on animal seizure models is similar, although not identical. The assumption, therefore, is that oxcarbazepine has its major effect in preventing repetitive firing of neurones by blocking voltage-dependent Na^+ channels (Rogawski and Porter 1990).

There may be some differences between oxcarbazepine and carbamazepine, in the way that high-voltage-activated (HVA) calcium currents are affected (Stefani et al, 1995), and in the modulation of

corticostriatal synaptic transmission (Calabresi et al, 1995). One trial (McKee et al 1994) demonstrated that oxcarbazepine could be added into a regime containing carbamazepine without necessarily provoking toxicity, and with occasional benefit.

Pharmacokinetics and interactions

Oxcarbazepine is rapidly and almost completely absorbed (96%) after oral dosing (Dam and Ostergaard 1995). After a rapid presystemic hydroxylation, it is excreted in the urine, 85% of each dose being excreted in the first 48 hours.

oxcarbazepine is immune to oxidative attack and it is the mono-hydroxy derivative of oxcarbazepine (OHCBZ) which accounts for the drug's pharmacological actions. Oxcarbazepine itself is only transiently present in the circulation (Theisohn and Heimann 1982). OHCBZ is approximately 40% bound to circulating plasma proteins; its elimination half-life ranges from 11 to 17 hours (Kristensen et al 1983).

Treatment with oxcarbazepine does not result in autoinduction of metabolism (Larkin et al 1991). In patients changed from carbamazepine to oxcarbazepine, steady-state plasma concentrations of concomitant phenytoin and valproate rose by 20-30%. oxcarbazepine had little effect on levels of established AEDs when used as add-on therapy suggesting an absence of important metabolic interference with these agents (McKee and Brodie 1994). The converse does not apply, however, as enzyme induction by phenobarbitone (Tartara et al 1993), phenytoin or carbamazepine may decrease OHCBZ concentrations. That the efficacy of oxcarbazepine relies on the production of OHCBZ may explain why twice-daily dosing may be adequate for a compound with a relatively short half life (Arnoldussen and Husmann 1991).

Efficacy

A number of clinical trials in which oxcarbazepine was substituted for carbamazepine have supported similar efficacy for the two compounds. In a double-blind randomised comparative trial with carbamazepine in 235 newly diagnosed patients (Dam et al 1989) no significant difference was found

between the two agents in terms of seizure control. Like carbamazepine, oxcarbazepine is not effective against absence seizures or myoclonic jerks (Friis et al 1993). Controlled trials in children are needed, although open studies support similar efficacy to that reported in adults.

Tolerability

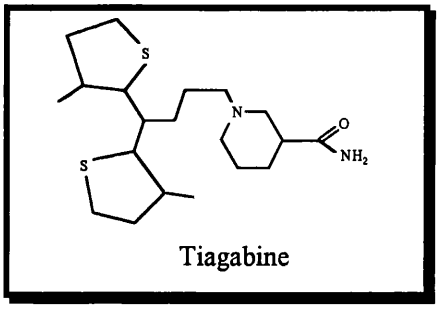
Side-effects associated with oxcarbazepine are similar to those produced by carbamazepine (Friis et al 1993) with dizziness, drowsiness, headache, nausea, vomiting and diplopia being the most prominent symptoms. Comparative studies (Dam et al 1989) have reported these to be less frequent and less severe than with carbamazepine. In addition, oxcarbazepine produces fewer rashes and perhaps fewer idiosyncratic reactions. Hyponatraemia, probably secondary to an antidiuretic hormone-like property, is more common with oxcarbazepine than with carbamazepine (Amelsvoort et al 1994). This can occasionally present clinically but is usually mild and asymptomatic. There is no evidence yet of teratogenesis with oxcarbazepine.

Oxcarbazepine has the potential to become a well established first-line antiepileptic drug when the current programme of research has been completed. Studies to date suggest that it is as effective as carbamazepine, but with lower propensity to induce idiosyncratic reactions and drug interactions. There is no evidence of teratogenicity so far, and the drug may have a less deleterious influence on cognitive function than carbamazepine. Placebo-controlled clinical trials are underway investigating the efficacy and safety of oxcarbazepine as adjunctive therapy in refractory epilepsy, as

monotherapy in newly diagnosed patients, in children and in the elderly. Finally, further studies on the basic mechanisms of action may dissect out important pharmacological differences between oxcarbazepine and carbamazepine.

Tiagabine

Tiagabine [(R-)-N-(4,4-di(3-methylthien-2-yl)but-3-enyl)nipecotic acid hydrochloride] like other nipecotic acid derivatives, inhibits GABA reuptake into both neurons and glial cells in rodents (Nielsen et al 1991). The large addition to the nipecotic acid molecule acts as a lipophilic anchor, helping the compound to cross the blood-brain barrier following oral administration.



Tiagabine

Mode of Action

Tiagabine is a potent GABA re-uptake blocker (Nielsen et al 1991), and the resultant increase in synaptic GABA, as demonstrated by microdialysis (Fink-Jensen et al 1992), is thought to account for tiagabine's anticonvulsant activity. As previously described, there are four GABA uptake transport mechanisms characterised, and nipecotic acid derivatives have been shown to be fairly specific for one of these (GAT-1), with some lesser effects on GAT-3 (Clark and Amara 1994).

Tiagabine only very weakly interacts with the BDZ receptor and the chloride ion channel of the $GABA_A$ receptor, and it does not appreciably bind to other neurotransmitter receptors (Rogawski and Porter 1990).

Preclinical testing

Tiagabine has been shown to protect against audiogenic seizures, PTZ-

induced seizures, and DMCM-induced seizures in mice. In rats, it has activity against PTZ picrotoxin and bicuculline-induced seizures as well as against amygdaloid kindling. It has some effect against photically induced seizures in baboons (Ostergaard et al 1995). Like vigabatrin, tiagabine increases the frequency and duration of spike wave discharges in Wistar rats (Coenen et al 1995)

In rat synaptosomal preparations, tiagabine inhibits the uptake of GABA into both neurons and glial cells (Braestrup et al 1990). At 100uM, tiagabine weakly (<20%) inhibits the binding of specific ligands for the dopamine D1 and D2, muscarinic, 5-HT2, $GABA_A$ and Glycine receptors. Radiolabelled tiagabine also showed saturable and reversible binding to rat brain membranes, which was inhibited by known inhibitors of H^3-GABA binding. There is no change seen in the transport of dopamine or noradrenaline. Radiolabelled tiagabine is not a substrate for the carrier process, and does not alter the rate of release of GABA from presynaptic neurones.

Interestingly, Honmou and colleagues (1995) have described an intriguing in-vitro example of pharmacological synergism. Gabapentin's action in stimulating non-synaptic GABA release is said to be potentiated on exposure to nipecotic acid. The mechanism by which this occurs is unknown, but it may be a harbinger of an important clinical finding.

Pharmacokinetics and interactions

Tiagabine is easily and rapidly absorbed following oral administration, with maximum concentrations occurring in most subjects less than 1 hour after

ingestion (Ostergaard et al 1995). In healthy volunteers pharmacokinetics are linear (Gustavson and Mengel 1995), with a half life between 5 and 8 hours (Brodie 1995). One quarter of each dose is metabolised and excreted in the urine, with the majority undergoing faecal excretion as two, as yet unidentified, metabolites (Ostergaard et al 1995).

Patients on a regime containing enzyme-inducing drugs would appear to metabolise tiagabine faster than untreated volunteers or patients on valproate monotherapy (Richens et al 1991, So et al 1995). Co-administration of tiagabine does not have an effect on the pharmacokinetics of concomitant AEDs (Richens et al 1995).

Clinical Studies

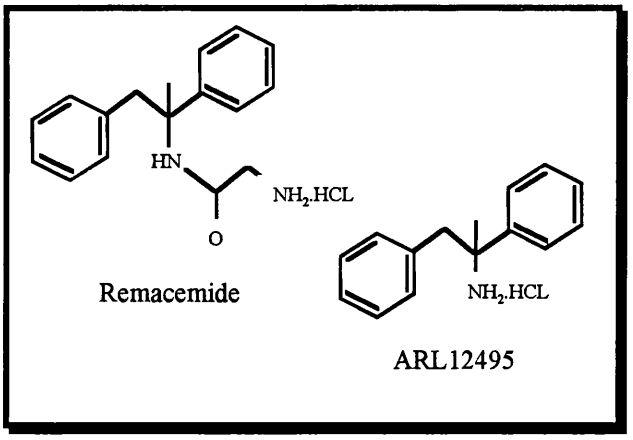
Published clinical studies are scanty, but the first fully published clinical study showed a positive effect (at least 25% reduction in seizure frequency) in 46 out of 94 patients with refractory localisation-related epilepsy (Richens et al 1995) during the open titration phase. Subsequent treatment during the placebo-controlled arm showed complex partial seizures to be at least halved in 26% of those undergoing double-blind testing against placebo. Of those with secondary generalised seizures, 63% had seizure frequency at least halved by tiagabine.

Tolerability

Tolerability seems to compare favourably with the established AEDs (Richens et al 1995). Ninety of the 94 recruits to the double-blind study (Richens et al

1995) reported some adverse events, unsurprisingly given that tiagabine dose was titrated to maximum tolerated dosage. Most adverse events were mild or moderate, with withdrawal being provoked in 14 patients. Of the ten considered retrospectively to be treatment associated, six complained of fatigue. During the double blind treatment phase, adverse events were slightly more common with placebo than with tiagabine treatment (62% and 50% respectively). Only one case of overdose has been reported (Leach et al 1995) which ended uneventfully despite the tiagabine serum level being thirty times greater than the mean serum level during treatment with the drug.

Remacemide



Remacemide is a novel antiepileptic agent which emerged from a drug discovery programme aimed at creating a molecule with a 3-dimensional structure similar to that of phenytoin (Rogawski and Porter, 1990). It is chemically unrelated to any other anticonvulsant, and is currently undergoing testing to assess its suitability for use as an add-on anticonvulsant drug. As well as being an anticonvulsant (Garske et al 1991), remacemide hydrochloride may be effective in preventing cell damage in the course of ischaemic injury (Bannan et al 1994) or Parkinson's disease (Greenamyre et al 1994).

Mode of action

At least part of the activity of remacemide arises from the active desglycinate metabolite, ARL12495XX. This metabolite has a longer half life than remacemide, and is commonly used in in-vitro experiments of anticonvulsant activity.

In keeping with the structural similarities with phenytoin, remacemide and ARL12495XX have been shown to inhibit sustained repetitive firing in cultured neurones (Cheung et al 1992) consistent with a blockage on fast sodium channels at concentrations which is suggested by the binding of both

remacemide and ARL12495 to the batrachotoxin-binding site of the sodium channel (Clark et al 1995).

Remacemide demonstrated no affinity for receptors to adenosine-1, $GABA_A$, benzodiazepines, glycine (Garske et al 1991), or the AMPA subtype of glutamate receptor (Hu and Davies 1994).

In-vitro studies have demonstrated no modification of evoked synaptic response or penicillin induced bursting in isolated rat hippocampal slices (Palmer et al 1992) by remacemide or ARL12495XX. Subsequent work in brain slices has demonstrated a change by ARL12495XX in K^+- and veratridine-mediated glutamate release (Srinivasan et al 1994).

A non-competitive antagonism at NMDA receptors (Palmer et al 1993) has also been demonstrated, which is more potent for ARL12495XX than for the parent compound, and an inhibition of NMDA-induced currents in cultured neurones was shown on exposure to both compounds (Subramaniam et al 1993). Other NMDA antagonists have been developed for use in seizures and ischaemic\anoxic brain injuries, but so far only remacemide has had sufficient tolerability to survive phase 1 testing.

Pharmacokinetics

The compound is a diphenyl-ethyl-acetamide derivative, and is rapidly and near-completely absorbed within two hours (Muir and Palmer 1991). In healthy volunteers, single doses up to 300mg were well tolerated, above which light-headedness and gastrointestinal upset became increasingly common. The elimination half life of remacemide in healthy volunteers is

around four hours, a rate that is independent of dose, with no evidence of any autoinduction. About 25% of the drug is excreted in the urine (Muir and Palmer 1991), mostly as glucuronide conjugates. The ubiquitous aminopeptidases are responsible for producing the main metabolite, ARL12495XX. This has a much longer half life (12-15 hours), and is thought to be, at least in part, responsible for the efficacy of remacemide. Levels of the desglycinate became detectable in volunteers at doses of remacemide above 300mg (Palmer et al 1992a). The elimination of ARL12495XX also demonstrates first order kinetics.

Preliminary evaluation in a small number of volunteers had suggested that the elimination of both remacemide and ARL12495XX was increased in those on monotherapy with enzyme-inducing anticonvulsants. There was some evidence that oral administration of remacemide hydrochloride had enzyme-inhibiting properties, which increased levels of concomitant carbamazepine.

In vitro studies (Riley et al 1995) had confirmed some effect of remacemide, albeit at high doses, on CYP3A4 and to a lesser extent CYP2C9. These enzymes are responsible for the oxidation of carbamazepine and phenytoin respectively.

Preclinical animal testing

In rodents, both the parent compound and ARL12495XX protected against MES-induced (Stagnitto et al 1990). After a single dose, this protection was sustained for up to 4 hours, longer than any of the established antiepileptic agents. After multiple doses, the anticonvulsant effect lasted for 8 hours,

longer than any of the established agents apart from phenobarbitone. The negative enantiomer is more potent at preventing MES induced seizures (Muir and Palmer 1991). No anti-seizure effect was found against seizures provoked by picrotoxin, strychnine, PTZ, or bicuculline (Muir and Palmer 1991).

Administration of remacemide to mice prevented NMDA-induced mortality. Interestingly, while ARL12495XX offered protection only against NMDA, remacemide itself also protected against kainate-induced mortality (Palmer et al 1992a).

Efficacy

Seizure frequency was reduced following administration of remacemide hydrochloride as add-on therapy in 28 patients with refractory epilepsy (Crawford et al 1992). One third of all patients had their seizure frequency cut by at least 50%, with a mean seizure reduction of around 33%. The results of much larger efficacy studies are awaited, these having 'unblinded observer' designs to compensate for the effects of pharmacokinetic interactions with other AEDs. More information on tolerability is also required.

Recurrent Materials and Methods

MATERIALS

Radioisotopes: γ-[^{14}C]-aminobutyric acid was obtained from DuPont (New England Nuclear).

Pharmaceuticals

Tiagabine was donated by Novo Nordisk

Vigabatrin was donated by Marion Merrell Dow

Remacemide was donated by Fisons Pharmaceuticals

Gabapentin was donated by Parke Davis

Chemicals: Ammonium dihydrogen orthophosphate ($NH_4H_2PO_4$), ammonium sulphate ($(NH_4)_2SO_4$), calcium chloride ($CaCl_2$), glacial acetic acid, D-glucose, hydrochloric acid (HCl), magnesium sulphate ($MgSO_4$), perchloric acid, phosphoric acid, potassium chloride (KCl), potassium dihydrogen orthophosphate (KH_2PO_4), sodium bicarbonate ($NaHCO_3$), sodium chloride (NaCl), di-sodium hydrogen orthophosphate (Na_2HPO_4), sodium hydroxide (NaOH), and sodium dihydrogen orthophosphate (NaH_2PO_4) were all obtained from Merck.

Para-aminobenzoic acid (PABA), γ-aminobutyric acid (GABA), 2-aminoethylisothironium bromide (AET), boric acid, bovine serum albumin (BSA), cytosine arabinoside (ARA-C), 3'5'-dibutyryl cyclic adenosine monophosphate (cAMP), dithiothreitol, ethylene diamine tetra-acetic acid

(EDTA), DNase I, gabaculline, L-glutamic acid, HEPES, insulin, α-ketoglutaric acid (α-KG), 3-mercaptopropionic acid (3-MPA), D,L,-norvaline, *o*-phthalaldehyde (OPA), poly-D-lysine, pyridoxal-5'-phosphate (PLP), soya bean trypsin inhibitor (SBTI), trypsin, and nipecotic acid were all obtained from Sigma Chemical Company.

Dulbecco's modified Eagle's medium (DMEM), Earle's balanced salt solution (EBSS), foetal calf serum (FCS), L-glutamine, horse serum (HS), minimal essential medium (MEM), penicillin, phosphate buffered saline (PBS), sterile culture water, and streptomycin were all obtained from Gibco BRL. Acetonitrile and methanol were obtained from Rathburn Chemicals Ltd. Coomassie Brilliant Blue G-250 protein assay dye reagent and Dowex AG50Wx8 ion exchange resin were from BIORAD.

A standard balanced salt solution (BSS) was used throughout the investigation of GABA uptake. Its composition was as follows: 136mM NaCl, 5mM KCl, 0.8mM $MgSO_4$, 2.6 mM $NaHCO_3$, 0.4mM KH_2PO_4, 0.34mM Na_2HPO_4, 1.3mM $CaCl_2$, 5.6 mM D-glucose, and 15mM HEPES. The solution was adjusted to pH 7.4 with NaOH and warmed to 37°C before use.

Equipment

Centrifugation: A Wifug haemicrofuge was employed for small volume samples and a refrigerated MSE Mistral 2L centrifuge for all other samples.

Electroshock stimulation: A Ugo Basile 7801 electroconvulsive therapy

(ECT) unit was used.

High performance liquid chromatography: For determination of amino acid concentrations, a Waters model 510 pump, a Waters WISP 710B injector, a Perkin Elmer LS-5 luminescence spectrophotometer, and a computer-based Jones Chromatography JCL-6000 integration package were used. Excitation and emission wavelengths were 330 and 440 nm respectively, with bandpasses of 15 and 20 nm.

Homogenisation: Where preservation of an enzyme was important a glass Potter Elvehjam vessel and motor powered teflon pestle were used. The alternative method employed a polytron homogeniser and included sonication in the homogenisation process. Small volume samples were homogenised by sonication alone in a MSE Soniprep 150.

Scintillation counting: A Canberra Packard 2000CA TRI-CARB liquid scintillation counter was used.

Spectrophotometry: Protein concentrations were assayed with a Philips Pye Unicam PU-8600 UV/vis spectrophotometer incorporating a PU-8605 cell programmer. (ELISA)

Statistical analysis: Statistical analysis was performed using the MINITAB statistical package (V8) on a Viglen 486DX microcomputer.

GENERAL METHODS

Animals used

ICR mice were supplied by Harlan Olac. Studies involving rats used the out-bred Sprague Dawley (SD) strain, which were supplied by Bantin and Kingman and again latterly by Harlan Olac. Neonatal and foetal animals required for cell culture techniques were supplied by the Joint Animal Facility at the University of Glasgow from a breeding colony of SD rats. Experimental animals were housed in the departmental animal unit. Animals were exposed to a controlled temperature and humidity environment throughout with a 12 hour light/dark cycle and had access to food and water *ad libitum*.

Brain tissue removal

Animals were sacrificed by a blow to the head followed by decapitation. The skin and tissues overlying the skull were incised and then removed. The point of a pair of bone cutters or scissors was inserted into the foramen magnum and the occipital bone incised, in either direction, in the dorsal plane. The occipital bone was then prised free of the underlying cerebellum. Next, the parietal bones of the skull were incised down either side, again in the dorsal plane, roughly at the level of the base of the brain. The parietal bones were prised clear of the brain surface taking care not to damage the underlying tissues. The meningeal membranes were cleared from the brain surface. Finally, the frontal bones were removed by a sharp fracture in the coronal plane just anterior to the olfactory lobes of the brain. Following severance of the optic nerves, the intact brain was removed with the aid of spatulas.

weighed where applicable, and stored appropriately at -70^{0}C until required. In studies involving analysis of discrete brain regions, the brain was carefully dissected in accordance with the method of Glowinski and Iversen (1966) and each region weighed prior to storage.

Determination of protein concentrations

Many of the assays reported in this thesis required accurate and reproducible analysis of sample protein content to enable quantifiable calculation of experimental results. The BIORAD method is a sensitive test of protein concentration of use particularly when small volume samples with low protein content are under investigation. The method relies on the colour change of a dye (Coomassie Brilliant Blue G-250) in response to protein concentration. Standards were prepared, in duplicate, over the range 0.5 - 2.0 μg/ml BSA. Samples of unknown protein concentration, also analysed in duplicate, were diluted into this range. BIORAD protein assay dye reagent was diluted 1:1 with water and added, in equal volume, to standards and samples alike. Following vortex-mixing, tubes were incubated at room temperature for 5 minutes and then read in the spectrophotometer. The intensity of the colour obtained was proportional to the amount of protein present and after construction of a standard curve, the protein content of samples could be determined. Results were corrected for dilution, averaged, and expressed in mg/ml.

Sample storage

Plasma and brain samples for drug assay were stored at -20^{0}C until analysis. Brain samples for the study of enzyme activities and neurotransmitter levels were stored at -70^{0}C until required.

DETERMINATION OF GABA-AMINOTRANSFERASE ACTIVITY

This method was devised from modifications of the methods of White and Sato (1978) and Larsson and co-workers (1986).

Reagents

All solutions required for enzyme assay were prepared in deionised water. An EDTA buffer was prepared weekly for sample preparation and stored at 4^{0}C. The buffer consisted of 0.1 mM EDTA, 0.5 mM dithiothreitol, and 0.1 mM KH_2PO_4. PLP was added daily as required (final concentration = 0.2 mM) and the buffer adjusted to pH 8.0 with 4 M NaOH. A [^{14}C]-GABA incubation medium was prepared every 2 - 3 months and stored at -20^{0}C. The incubation medium consisted of 0.68 mM GABA (specific activity = 1.46 mCi/mmol), 1.8 mM EDTA, and 200 mM K H_2PO_4 with the pH adjusted to 6.9 with 1 M NaOH.

Sample preparation

Neurological tissue was thawed and homogenised in 4 volumes (v/w) of EDTA buffer. All samples were centrifuged at 2000 rpm for 20 minutes at 4^{0}C. The resultant supernatant was decanted and its protein content determined, in duplicate, by the BIORAD method (see above: *General Methods - determination of protein concentration*). The volume of the remaining

supernatant was adjusted with EDTA buffer to give a final protein concentration of 1 mg/ml.

Assay for enzyme activity

A 50 μl volume of the adjusted supernatant was added to 25 μl of 0.68 mM α-Ketoglutarate (α-KG) and 25 μl of the [^{14}C]-GABA incubation medium to give a final assay volume of 100 μl. Assays were performed in duplicate with a blank assay included for each sample by replacing the α-KG with 25 μl water. All samples were vortex-mixed for 10 seconds and then incubated for 60 minutes at 37^{0}C. The reaction was terminated by the addition of 10 μl 2M HCl followed immediately by vortex-mixing for 10 seconds. The incubation mixtures were transferred to the surface of a resin in small disposable ion-exchange columns (Dowex AG50Wx8, pre-washed with deionised water, 0.5 x 3.0 cm, in 9 inch glass Pasteur pipettes plugged with a glass bead). Radioactive products were eluted directly into glass scintillation vials using 3 portions of 0.5 ml water. Each portion was placed in the original incubation tube and transferred to the column with the same pipette used to transfer the incubation mixture. Twelve ml of Picofluor 40 scintillation fluid was added to each vial and the disintegrations per minute (dpm) were counted for 10 minutes by liquid scintillation counting.

Calculations

The radioactive content of samples was analysed in comparison to the dpm obtained from standard solutions containing known amounts of radioligand.

Results were corrected for background and blank sample counts and quantified in relation to protein content and reaction time. Enzyme activities were expressed as nmol/min/mg protein.

DETERMINATION OF AMINO ACID CONCENTRATIONS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

This method for the measurement of GABA, glutamate and glutamine was devised from a modification of the method of Durkin and colleagues (1988).

Reagents

Stock solutions of GABA (1 mg/ml in distilled water) were prepared monthly, stored at 4^{0}C, and diluted to working standard solutions daily as required. The derivatization reagent mixture (OPA-3-MPA) was prepared weekly by dissolving 50 mg OPA in 4.5 ml methanol and 0.5 ml borate buffer then adding 50 µl 3-MPA with the mixture being stored at 4^{0}C in the dark. The borate buffer was made weekly by adjusting 0.5 M boric acid to pH 9.5 with 1 M NaOH.

Mobile phase

Glutamate and glutamine concentrations were determined in mobile phase consisting of 75:25 (v/v) 0.57 M acetate buffer (pH 3.75, containing 100 mg/l EDTA) / acetonitrile. For GABA analysis the mobile phase was 60:40 (v/v) 0.2 M acetate buffer (pH 3.80, containing 100 mg/l EDTA) / acetonitrile. Acetate buffers were prepared by adding 32.8 ml (0.57 M) or 11.5 ml (0.2 M) glacial

acetic acid to 925 ml of water, adjusting the pH with 3 M NaOH and diluting to 1 litre with water. Flow rates were 1.0 ml/min throughout.

Calibration

Calibration curves (in water) were constructed for glutamate (0.5 - 5.0 μg/ml), glutamine, and GABA (2.0 - 20 μg/ml) and were seen to be linear ($r \geq 0.930$) in all cases. Limits of detection for both glutamate, glutamine and GABA were found to be 5 ng/ml in a 50 μl sample. Intra- and inter-assay variations for GABA were calculated at 2.8% and 7.9% respectively, while for glutamate they were 3.4% and 8.7%, and for glutamine, 3.8% and 8.2%.

Sample preparation

Whole brains were homogenised in 10 volumes (v/w) of 1% perchloric acid and an aliquot taken for determination of protein content by the BIORAD method (see above: *General Methods - determination of protein concentration*). All samples were centrifuged at 2000 rpm for 5 minutes, the supernatant decanted and diluted 1/10 with water prior to derivatization.

Derivatization

A 50 μl aliquot of the diluted supernatant was reacted with 200 μl methanol, 200 μl borate buffer, and 50 μl OPA-3-MPA solution. D,L-norvaline (50 μl) was added as an internal standard to give a total reaction volume of 550 μl. Reaction mixtures were vortexed and allowed to stand at room temperature for 4 minutes prior to injection of 10 μl onto the column.

Calculations

Amino acid concentrations were calculated by comparison of peak height ratios of analyte to internal standard and quantified in relation to the wet weight of tissue for brain samples and the protein concentration for cell culture samples. Results were expressed as μg/g protein.

DETERMINATION OF GLUTAMIC ACID DECARBOXYLASE ACTIVITY

This method was devised from modifications of the methods of Kocchar and colleagues (1989), Wolf and Klemisch (1991), and Chakraborty and co-workers (1991).

Reagents

A sodium phosphate-AET buffer was prepared weekly for sample preparation and stored at 4^0C. The buffer consisted of 0.1 M Na_2HPO_4 and 1 mM AET. The buffer pH was adjusted to 7.0 with 0.1 M NaH_2PO_4. An incubation medium was prepared daily as required and consisted of 50 mM L-glutamic acid, 250 μM PLP, 0.4% 2-mercaptoethanol, and 57 μM gabaculline.

Sample preparation

Whole brains were thawed and homogenised in 10 volumes (v/w) sodium phosphate-AET buffer. Samples were then centrifuged at 2000 rpm for 10 minutes. The supernatant was decanted and an aliquot taken for

determination of protein content by the BIORAD method (see above: *General Methods - determination of protein concentration*).

Assay for enzyme activity

Incubation medium (100 µl) was added to each of two 100 µl aliquots of supernatant per sample. The reaction in one aliquot (Zero-time) was terminated immediately while the other (test) was allowed to continue for a period of 60 minutes at 37^{0}C. Termination was performed in both cases by the addition of 100 µl 1% perchloric acid. Terminated blank and test reaction mixtures were diluted 1/10 with water and assayed for GABA content by high performance liquid chromatography (See above: *HPLC - derivitization*).

Calculations

Enzyme activity was calculated by subtraction of the zero-time GABA concentration from the test GABA concentration to give a value for GABA production during the reaction period. Results were quantified in relation to both reaction time and protein concentration and were expressed as nmol/min/mg protein.

PRIMARY CULTURE OF CEREBRAL CORTICAL ASTROCYTES

This method was devised from modifications of the methods of Larsson and co-workers (1981) and Bender and Hertz (1984).

Reagents

A culture medium was prepared which consisted of DMEM supplemented with 20% (v/v) HS, 2.5 mM L-glutamine, 50 I.U./ml penicillin, and 50 μg/ml streptomycin. The medium was further supplemented with 0.25 mM cAMP, where indicated, to facilitate differentiation of the cells. The medium was prepared under sterile conditions and filter sterilised through a 0.2 μm pore filter prior to use and/or storage. Media were stored sterile at 4^0C for up to 5 days.

Tissue preparation

Tissue for cell isolation was removed under aseptic conditions. One day old rat pups were decapitated and the skin overlying the skull was peeled away. The entire skull surface was removed by inserting the point of a pair of scissors into the foramen magnum and incising the skull down either side in the dorsal plane, taking care not to damage the underlying cortex. The cerebral cortices were removed from either hemisphere with a sharp pinch between the points of a pair of curved watchmaker's forceps. The removed tissue was placed in a 55 mm^2 culture dish containing DMEM and, with the aid of a dissecting microscope, the olfactory bulbs, basal ganglia, hippocampal formations and meninges were removed. The dissected neopallia were then transferred to a sterile universal tube containing 6 ml of DMEM prior to cell isolation.

Cell isolation

The dissected neopallia were cut into small cubes (0.5 mm^3) by two passes (at 90°) in a McIlwain tissue chopper. The chopped tissue was transferred to a sterile filter (80 μm nylon mesh) and the filtrate collected in a sterile beaker. The chopped material was washed through the filter with culture medium to give a final volume of 3 ml per brain. A sterile plastic pipette was used to aid this process. The filtrate was passed through a sterile needle (BD Microlance 21G 0.8 x 40) three times to separate the cells. The volume of the resulting suspension was adjusted with culture medium to allow a 3 ml aliquot per petri dish with a ratio of 1 brain to 3 dishes. A 3 ml volume of the final cell suspension was plated onto 55 mm^2 Falcon Primaria culture dishes.

Culture maintenance

The cultures were maintained at 37°C in an environment of 95% O_2 / 5% CO_2 with a humidity of ≥ 90%. The culture medium (3 ml) was replaced every 3 - 4 days throughout. The HS concentration was reduced to 10% at the first medium change with a final reduction to 5% at the second change. The HS concentration remained at 5% thereafter. Once the cells reached confluence (usually after 14 days in culture) the medium was supplemented with 0.25 mM cAMP. At this stage penicillin and streptomycin were omitted from the culture medium due to possible interference with subsequent experimental procedures. Cultures were seen to be fully mature and ready for use between day 21 and day 24 and were viable for up to 42 days.

[^{14}C]-GABA UPTAKE INTO CULTURED ASTROCYTES

This method was devised from modifications of the methods of Larsson and co-workers (1981) and Yu and colleagues (1984).

Reagents

A standard balanced salt solution (BSS) was used throughout the investigations of [^{14}C]-GABA uptake. Its composition was as follows: 136 mM NaCl, 5 mM KCl, 0.8 mM $MgSO_4$, 2.6 mM $NaHCO_3$, 0.4 mM KH_2PO_4, 0.34 mM Na_2HPO_4, 1.3 mM $CaCl_2$, 5.6 mM D-glucose and 15 mM HEPES. The solution was adjusted to pH 7.4 with 1 M NaOH and stored, at 4^{0}C, for up to 1 week. BSS was warmed to 37^{0}C prior to use.

Culture preparation

Cultures for investigation were removed from the incubator and the existing culture medium aspirated. Cultures were washed twice (2 x 1 ml neurones, 2 x 2 ml astrocytes) with BSS (37^{0}C) before being returned to the incubator in a further volume of BSS (2 ml neurones, 3 ml astrocytes) for an equilibration period of 20 minutes.

[^{14}C]-GABA uptake procedure

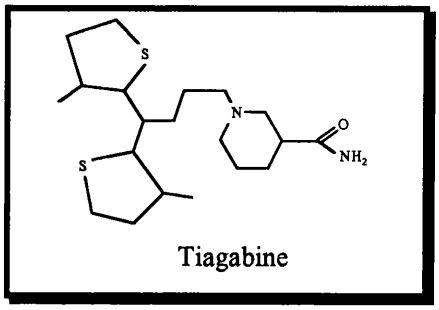
The pre-washed cultures were removed from the incubator and the existing BSS aspirated. This solution was replaced by BSS (1 ml neurones, 2 ml astrocytes) containing the drug concentrations appropriate to the individual experiment. Control plates received BSS alone. All culture plates were

returned to the incubator for a further period of 1 hour. After the incubation period, a further 1 ml of BSS (with appropriate control/drug treatment) containing 150 μM [^{14}C]-GABA (specific activity = 1 mCi/mmol) was added to each plate. Incubation was allowed to continue for 5 minutes before the cultures were washed with 5 volumes (2 ml) of warmed BSS. Cells were finally removed from the plates by scraping in 1.0 ml 1 M NaOH. Aliquots were taken for protein determination by the BIORAD method (see above: *General Methods - determination of protein concentration*) and liquid scintillation counting in 8 ml of Picofluor 40 scintillation fluid.

Calculations

Liquid scintillation counting was employed to analyse GABA uptake in individual cultures in comparison to the dpm of standard solutions containing known amounts of radioligand. Results were quantified by the relation of GABA uptake to the protein concentration and expressed as pmol/min/mg protein in individual cultures. **Tiagabine**

Tiagabine [(R-)-N-(4,4-di(3-methylthien-2-yl)but-3-enyl)nipecotic acid hydrochloride] like other nipecotic acid derivatives, inhibits GABA reuptake into both neurons and glial cells in rodents (Nielsen et al 1991). The large addition to the nipecotic acid molecule acts as a lipophilic anchor, helping the compound to cross the blood-brain barrier following oral administration.



Tiagabine

Clinical investigation into the interactions between remacemide hydrochloride and phenytoin, carbamazepine, and sodium valproate

Clinical evaluation of the pharmacokinetic interactions of remacemide with established AEDs

Standard development and clinical assessment of new AEDs requires that they are initially used and assessed as add-on therapy. The first impression gained of any new drug will therefore be affected by any pharmacokinetic or pharmacodynamic interactions that occur when the drugs are used alongside established AEDs. In remacemide's case, early assessment is doubly important because use in man involves production of an active desglycinyl metabolite, ARL12495XX.

In the following set of studies three patient populations undertook a trial of oral remacemide hydrochloride. Patients studied were on monotherapy with either valproate, carbamazepine or phenytoin. The trial protocol, common to all three groups will first be described. The demographics and pharmacokinetic responses of each group will then be dealt with separately.

Protocol

The study had a double-blind, random order, placebo-controlled, crossover design, preceded by an open, single-dose treatment phase. Patients continued to take their baseline AED in their usual dose throughout. One week following a screening visit, each patient received a single dose of 300mg remacemide hydrochloride. Plasma levels of baseline AED, CBZ-E (where applicable), remacemide, and ARL12495XX, were measured 0, 0.5, 1,

1.5, 2, 4,6, 8,10, 12, 24, and 48 hours after dosing.

One week later, patients entered the first arm of chronic treatment, receiving either remacemide hydrochloride or matched placebo (100mg twice daily on day 1, 200mg twice daily on day 2, and 300mg twice daily thereafter). The total treatment period was 14 days, after which remacemide hydrochloride was stopped to allow measurement of washout concentrations at the same times after dosing as before. Seven days later, the second treatment phase was commenced and the whole procedure was repeated. Morning pre-dose (trough) samples were taken on the 5th, 12th and 15th day after initiation of treatment.

Initially, as with those patients on enzyme inducing AEDs, valproate-treated patients were given remacemide at a dose of 300mg BD during the multiple dosing phase. Recognising the possibility of a pharmacokinetic interaction between remacemide and valproate, the protocol allowed for a dose reduction to 150mg BD in the event of any perceived adverse event. After four out of the first nine patients on concomitant valproate had required reduction in remacemide dose, the dose of remacemide hydrochloride was reduced to 150mg BD throughout the multiple dosing phase. The doses preceding forty-eight hour plasma level monitoring remained 300mg.

All blood samples were taken into heparinised tubes from a cannulated forearm vein, which was kept patent between aspirations with normal saline. On each occasion, the first 1ml withdrawn was discarded, and the subsequent 15ml were chilled until centrifugation. All samples were spun at 3,000 rpm for 10 minutes, and the separated plasma frozen at -4^{o}C for batch analysis.

The study was approved by both the West Ethical Committee in Glasgow and the Research Ethics Committee in Cardiff. Written informed consent was obtained from each participant

Assays

All assays were carried out by the laboratories at Astra Pharmaceuticals. Remacemide and its desglycinyl metabolite were quantified by high performance liquid chromatography (HPLC). This was a modification of a previously reported method (Flynn and O'Brien 1992), adjusted to allow automated sample preparation and improve selectivity. The method involved solid phase extraction followed by separation on a reverse phase HPLC system utilising a octadecyl (C-18) HPLC column, an acetonitrile based eluent, and ultraviolet (uv) detection at 210 nm. Limits of quantification for the two analytes were 10 ng/ml. Only samples from the active leg of the double-blind phase were analysed for remacemide and ARL12495XX. Carbamazepine, phenytoin and CBZ-E were measured by liquid-liquid extraction of plasma followed by reverse phase HPLC utilising a C8 column, a methanol-based mobile phase, and uv detection at 210nm. Sodium valproate was extracted from acidified plasma using chloroform and was analysed by flame-ionised chromatography on DB-WAX.

Pharmacokinetics

The following non-compartmental pharmacokinetic parameters were computed where appropriate for valproate, phenytoin, carbamazepine and CBZ-E for all three phases of serial blood sampling:

- area under the concentration-time curve over a dosing interval (AUC_{0-t}) calculated using the linear trapezoidal method
- peak concentration (Cmax) over the dosing interval
- trough/pre-dose concentration (Cmin) over the dosing interval
- time to maximum concentrations (Tmax)
- Cmin 5, 12, and 15 days after initiation of multiple dosing with remacemide hydrochloride or placebo

For remacemide hydrochloride and ARL12495XX, the parameters calculated following single dose and multiple dosing were:

- Cmax
- Tmax
- AUC after single dose was extrapolated to infinite time (AUC_{∞}), calculated from AUC = AUCt + Ct/kel where AUCt = area under the curve up to the last point at which the concentration could be quantified, and kel = the terminal phase plasma elimination rate constant. After multiple doses AUC was calculated over a 12-hour dosing interval (AUC_{0-12h})
- Elimination half-life, ($t_{½}$) after the single dose and during washout of the multiple dose remacemide hydrochloride treatment phase. This was calculated from $t_{½}$ = 0.693/kel.
- Cmin 5, 12, and 15 days into remacemide hydrochloride treatment.

Statistics

Statistical comparisons of the pharmacokinetic parameters obtained for baseline AEDs and CBZ-E at the end of the two multiple dose phases were compared using an analysis of variance with treatment, period, order, group and patients as factors. Logarithmically transformed data were used for analysis of the AUC, Cmax and Cmin comparisons. Analysis of the trough concentrations used ANOVA for the three concentrations per patient (5, 12, and 15 days after initiation of multiple dosing) with factors of treatment, period, sequences, day number and patient. Single and multiple dose phases were compared using a non-parametric procedure, the Wilcoxon matched pairs signed rank test. A probability less than 5% indicated statistical significance.

Results in carbamazepine-treated patients

Patients

Of the 14 patients recruited (Table 2), 10 completed the study as per protocol. One patient (C018) withdrew a few hours following administration of the single remacemide hydrochloride dose because he disliked intravenous cannulation. Another (C021) pulled out 5 days after single dose administration because of an intercurrent viral illness. One patient (C024) was withdrawn from the study because of suspected poor compliance, while a fourth (C016) had the dose of remacemide hydrochloride halved following the onset of adverse events suspected to be due to the study drug. This last patient's data

Table 2

Demographic characteristics of carbamazepine-treated patients.

Patient	Sex	Age (years)	Seizure type	CBZ dose (mg/L)	Dosage interval (hours)
C 001	M	36	CP, CPGTC	600	8
C 002	M	47	SP, SPGTC	800	8
C 003	M	48	CP, CPGTC	800	12
C 016	F	65	CP, CPGTC	800	12
C 017	F	43	CP, CPGTC	800	12
C 018	M	46	SGTC	400	12
C 019	M	56	SP, SPCP	600	8
C 020	M	39	CP, CPGTC	600	24
C 021	F	57	SGTC	400	12
C 022	F	46	SP, SPCP, SPCPGTC	1200	12
C 023	M	57	SP, SPCP, SPCPGTC	800	12
C 024	F	51	PGTC	800	12
C 025	F	40	CP, CPGTC	1200	24
C 027	F	40	PGTC	1600	12

CBZ= carbamazepine CP=complex partial, CPGTC=complex partial with secondary generalisation, SP=simple partial, SPGTC=simple partial with secondary generalisation, SGTC=secondary generalised tonic clonic seizures, SPCP=simple partial evolving into complex partial seizures, PGTC=primary generalised seizures.

are included in the summary of adverse events, but not in the pharmacokinetic analysis.

Carbamazepine pharmacokinetics

There were no changes in mean carbamazepine pharmacokinetic parameters following a single dose of remacemide hydrochloride (Figure 6 and Table 3). Following 14 days' treatment, however, the mean $AUC_{0\text{-}12h}$ of carbamazepine was increased by 22% ($p = 0.12$), the mean Cmax by 27% ($p = 0.07$), and the mean Cmin by 22% ($p = 0.29$). Unsurprisingly, Tmax was unchanged following both single and multi-dose remacemide hydrochloride.

Comparison of mean trough carbamazepine levels 5, 12, and 15 days after the start of active treatment (40.1, 34.4 and 40.4 umol/L respectively) with those on placebo (32.7, 30.5 and 34.5 umol/L respectively) showed a statistically significant increase ($p=0.0013$). Four patients had at least one of the pharmacokinetic parameters of carbamazepine increased by more than 30% during the remacemide hydrochloride treatment phase. None of these patients, however, reported any symptoms suggestive of carbamazepine toxicity.

CBZ-E pharmacokinetics

The mean AUC, Cmax, and Cmin for CBZ-E (Table 3) were not significantly altered by concomitant remacemide hydrochloride following single or multiple dosing (Figure 7). After 14 days' treatment, two patients had an increase in AUC or Cmax of more than 30%, one being a rise in AUC of 177% and in

Figure 6

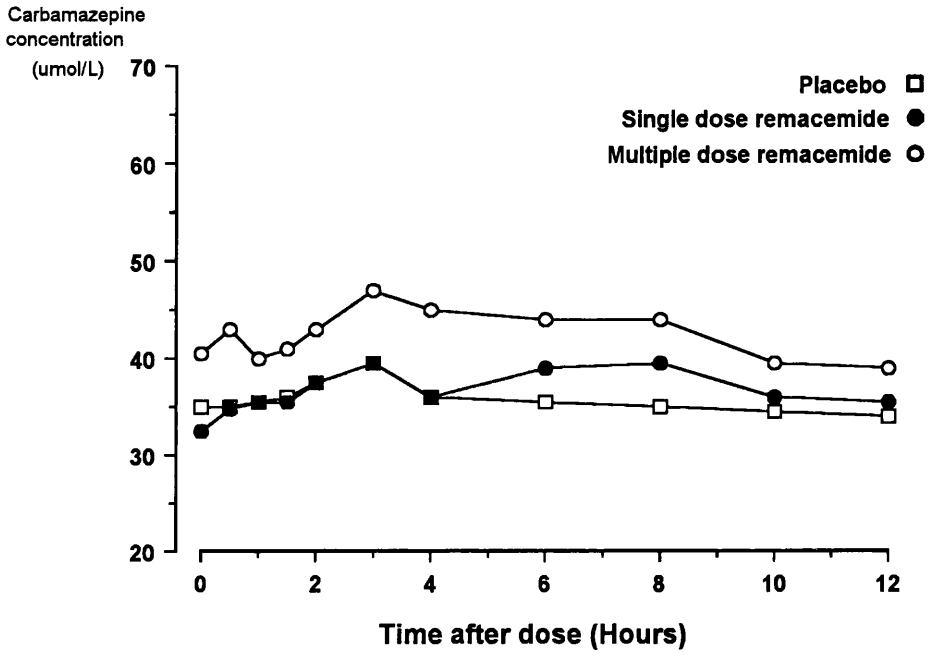


Figure 6: Mean plasma concentrations of carbamazepine in 10 patients with epilepsy over a 12 hour period following single (300mg) or multiple (300mg BD for 14 days) dosing with remacemide hydrochloride or placebo.

Table 3

Mean carbamazepine pharmacokinetic parameters (SD) after single and multiple doses of remacemide hydrochloride and placebo in 10 epileptic patients.

	AUC (umol.hr.l^{-1})	Cmax (umol.l^{-1})	Tmax (hours)	Cmin (umol.l^{-1})
Placebo	367.8 (135.2)	40.5 (8.7)	4.8 (4.6)	34.7 (8.2)
Single dose	392.3 (161.1)	43.3 (10.1)	7.1 (4.2)	34.0 (7.2)
Multiple doses	425.4 (156.9)	50.6 (12.2)	5.1 (3.5)	40.8 (11.6)

Figure 7

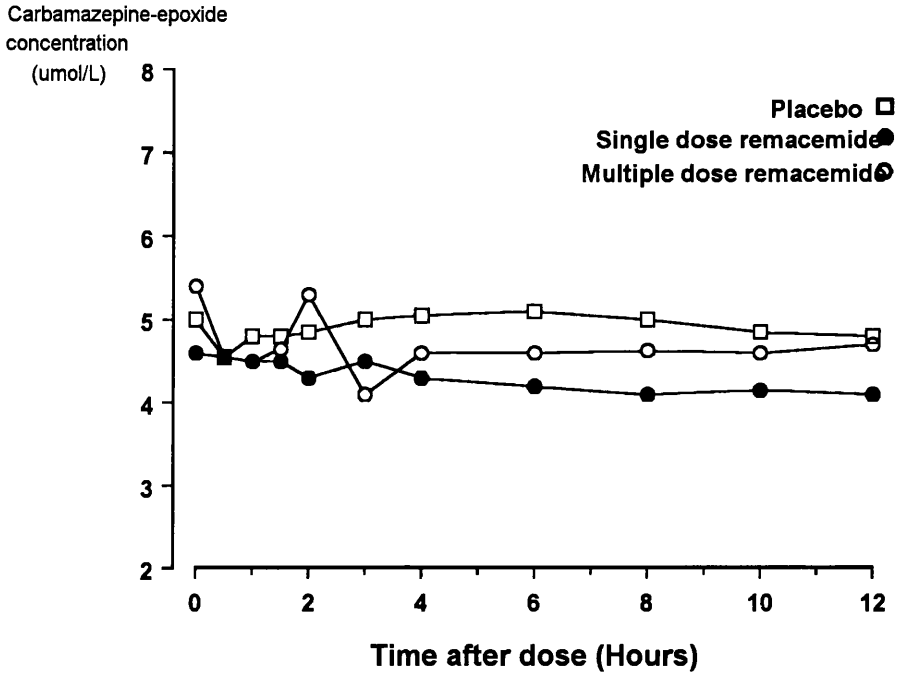


Figure 7: Mean plasma concentrations of carbamazepine10,11-epoxide in 10 patients over a 12 hour period following single (300mg) or multiple (300mg BD for 14 days) dosing with remacemide or placebo.

Table 4

Mean carbamazepine 10,11 epoxide pharmacokinetic parameters (SD) after single and multiple dose of remacemide hydrochloride and placebo in 10 epileptic patients

	AUC (umol.hr.l^{-1})	Cmax (umol.l^{-1})	Tmax (hours)	Cmin (umol.l^{-1})
Placebo	53.8 (32.7)	5.6 (2.6)	6.2 (5.1)	5.0 (2.4)
Single dose	46.7 (26.9)	5.0 (2.2)	4.6 (4.6)	4.5 (2.2)
Multiple doses	48.4 (27.5)	5.9 (2.4)	5.2 (4.6)	4.9 (2.2)

Cmax of 153%. These patients, however, remained symptom-free. There was no significant difference in Tmax following acute or chronic remacemide hydrochloride dosing. Comparison of mean trough CBZ-E levels 5, 12, and 15 days after the start of active treatment (5.1, 4.4 and 5.0 umol/L respectively) with those during placebo treatment (4.9, 4.1, and 5.0 umol/L respectively) showed no significant differences ($p = 0.62$).

Remacemide and ARL12495XX pharmacokinetics

Mean plasma concentrations following single and multiple dosing are illustrated for remacemide in Figure 8, for ARL12495XX in Figure 9, and for both in table 5. As anticipated from a drug with a considerably shorter half-life than dosing interval, there was little carry-over of remacemide from dose to dose, and the steady-state profiles attained were at only slightly higher levels than those following the single dose (Figure 8 and Table 5). For ARL12495XX, however, consistent with its longer terminal half life, there was a greater carry-over during multiple dosing, and the maximum concentrations attained at steady-state were approximately twice those following the single dose (Figure 9 and Table 5). Steady state peak (Cmax) to trough (Cmin) oscillations were much smaller for ARL12495XX than for remacemide.

With both remacemide and ARL12495XX, there was good predictability of multiple dose profiles compared with single dose profiles based on linear superposition of the concentration data and comparisons of AUCs following

Table 5

Mean pharmacokinetic parameters (SD) of remacemide hydrochloride and ARL12495XX in 10 carbamazepine treated patients following acute and chronic dosing.

	Cmax ($ng.ml^{-1}$)	**Cmin** ($ng.ml^{-1}$)	**Tmax** (hours)	**AUC**** ($ng.hr.ml^{-1}$)	**t1/2** (hours)
Single Dose					
Remacemide	783 (229)	NA	1.5 (1.0)	2266 (1344)	3.6 (1.3)
ARL12495XX	30.2 (7.6)	NA	2.0 (0.7)	395 (125)	10.4 (0.6)
Multiple Dose					
Remacemide	1006 (411)	60.9 (74.8)	1.1 (0.4)	2644 (1376)	3.5 (1.4)
ARL12495XX	64.8 (23.2)	25.2 (7.9)	1.6 (0.9)	427 (108)	11.2 (4.1)

**AUC_{∞} for single dose profiles, $AUC_{0\text{-}12h}$ for multiple dose profiles
NA = not applicable

Figure 8

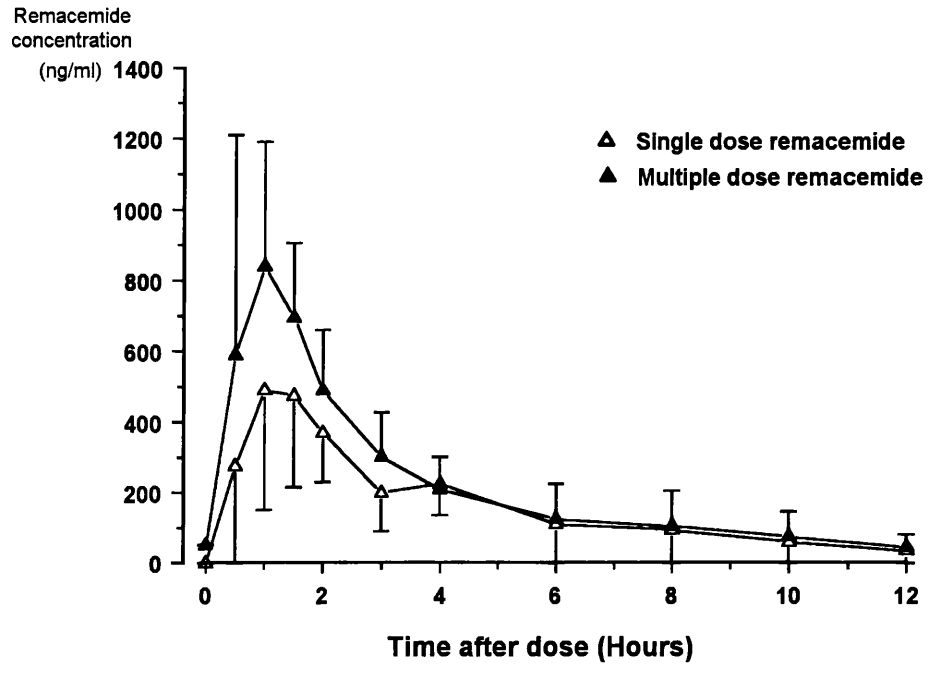


Figure 8: Mean (+/-SD) single dose and steady state plasma concentrations of remacemide in 10 patients taking carbamazepine who received a single dose (300mg) or multiple doses (300mg BD for 14 days) remacemide hydrochloride.

Figure 9

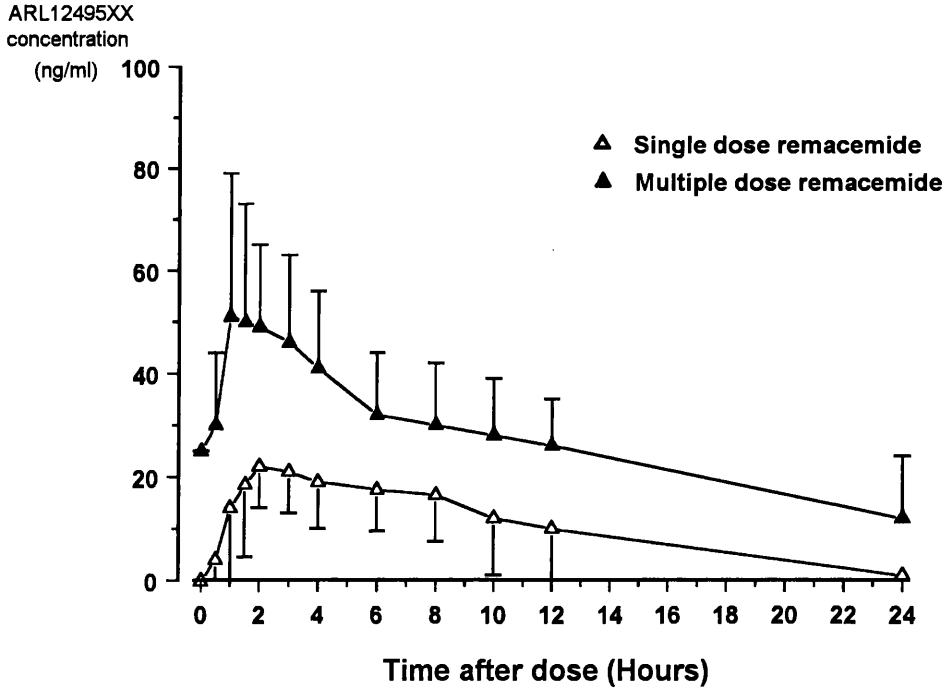


Figure 9: Mean (+/-SD) single dose and steady state plasma concentrations of ARL12495 in 10 patients taking carbamazepine who received a single dose (300mg) or multiple doses (300mg BD for 14 days) remacemide hydrochloride.

single and multiple dosing. This suggests that there was no autoinduction of remacemide or ARL12495XX metabolism.

Adverse events

No major adverse events were reported, and no patients were withdrawn from the study due to adverse events, although one patient (C016) had his dose of remacemide hydrochloride halved 4 days into the multiple-dose phase because of dizziness. Overall, more events were reported while patients were on placebo (36 adverse events) than following remacemide hydrochloride treatment (26 events reported). Similar numbers of patients reported adverse events following multiple dosing with active treatment (10 patients) as were reported with placebo (9 patients). There were similar numbers of central nervous system events (4 on remacemide hydrochloride versus 3 on placebo), and gastrointestinal symptoms reported were equal on both treatments (2 each).

Discussion

Carbamazepine is a well-known inducer of the hepatic P450 mono-oxygenase enzymes (Brodie 1992). This results in marked intra- and inter-individual variations in serum concentrations of other antiepileptic drugs during polypharmacy, an unpredictability which is exacerbated by variable autoinduction of metabolism (Levy and Wurdland 1995). In addition, many other drugs have been shown to interact pharmacokinetically with carbamazepine (McKee and Brodie 1994). A major pathway involves oxidation

of carbamazepine to the 10,11-epoxide (CBZ-E), which is itself biotransformed by the enzyme epoxide hydrolase to the inert dihydrodiol (Eichelbaum et al 1985). These two processes provide a target for interactions between carbamazepine and other antiepileptic drugs (Brodie et al 1983, Macphee et al 1988, McKee et al 1992). Changes in the epoxide concentration may be important because this CBZ-E contributes to the efficacy and adverse events associated with carbamazepine treatment (Gillham et al 1988). In one study the majority of patients on co-medication with remacemide hydrochloride had dose-related increases in trough concentrations of carbamazepine, necessitating a reduction in carbamazepine dose in a few patients (Clark et al 1995).

Like carbamazepine, remacemide is also eliminated almost exclusively by metabolic transformation. Apart from the active metabolite ARL12495, which is formed by the ubiquitous aminopeptidase enzymes, there are a number of oxidative biotransformation products. In addition, remacemide hydrochloride undergoes direct glucuronidation to form a carbamoyl glucuronide metabolite, an important pathway in man (Clark et al 1995).

Following multiple dosing with remacemide hydrochloride, there was an overall small inhibition of carbamazepine metabolism during active treatment compared with placebo. Three patients demonstrated a rise in carbamazepine trough levels of >30%. There were no significant changes overall in CBZ-E concentrations during treatment with remacemide hydrochloride, although there were marked differences in individual response with one patient exhibiting an increase in CBZ-E level of more than 100%. No patients had any

clinical sequelae.

These findings are consistent with the in-vitro experiments using 6-B-hydroxylation of testosterone as a marker of CYP3A4 activity (Riley - manuscript in preparation), which showed that remacemide is an inhibitor of cytochromal activity associated with this isoform. Since the concentrations of remacemide required in the in-vitro mixture to achieve inhibition of CYP3A4 are in excess of those reached in vivo, it is fair to assume that any increase in carbamazepine concentrations will be modest. Remacemide has not been shown to affect epoxide hydrolase in vitro, in keeping with the findings in this study.

This study also offered the opportunity to provide a pharmacokinetic profile of remacemide and ARL12495XX in enzyme-induced patients. For both compounds, the multiple dose profile was consistent with that predicted from the single dose, indicative of linear disposition and the absence of autoinduction of metabolism. The terminal half life of remacemide was similar to that found in previous clinical studies in human volunteers (Figure 10), whereas that of ARL12495XX in enzyme-induced patients was shorter than in untreated healthy volunteers (Clark et al 1995). Exposure to remacemide based on AUC values was around 60% of that reported previously in non-induced subjects taking the same dose of the drug, while that of ARL12495XX was about 30% (Figure 10).

The most common adverse events reported with remacemide hydrochloride were dizziness and mild to moderate gastrointestinal upset (Clark et al 1995).

Figure 10

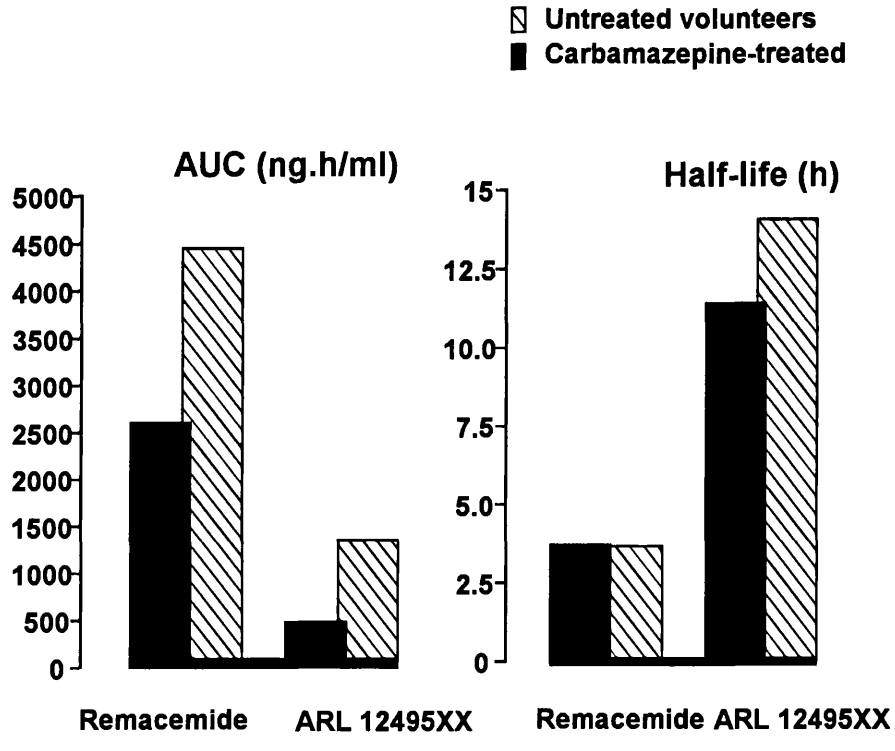


Figure 10: Mean pharmacokinetics of remacemide and ARL12495 in patients pretreated with carbamazepine versus healthy untreated volunteers (Data on file Astra Pharmaceuticals)

There were no adverse events precipitating withdrawal from the study, although one patient reported dizziness, necessitating a decrease in remacemide hydrochloride dose. A greater number of adverse events were reported during the placebo phase. In healthy, untreated volunteers, the AUC following similar dosing with remacemide hydrochloride (600mg/day) was between two and three times higher than the doses used in patients taking carbamazepine in this study. Consequently, 600mg per day is unlikely to be the maximum tolerated dose of remacemide hydrochloride in patients receiving treatment with carbamazepine.

There was considerable inter-patient variability in the pharmacokinetic response following the introduction of remacemide hydrochloride suggesting differences in individual susceptibility to the interaction. Although vigilance should be exercised in adding remacemide hydrochloride to antiepileptic drug regimes containing carbamazepine, it is questionable whether a reduction in carbamazepine dosage will be required in most patients. Since the presence of carbamazepine will result in lower bioavailability of remacemide and ARL12495XX, patients pre-treated with an enzyme-inducer such as carbamazepine will require higher doses of remacemide hydrochloride than non-induced patients. In addition, the remacemide concentration can be expected to rise when carbamazepine is withdrawn. Since remacemide and ARL12495XX exhibit predictable and linear kinetics in carbamazepine patients, with no evidence of autoinduction, there should be little need for routine therapeutic monitoring of either drug in this clinical setting. The mutual interaction between carbamazepine and remacemide hydrochloride is

predictable and modest, and should not present a barrier to their clinical use in combination.

Results from phenytoin-treated patients

Patients

Of the 11 patients recruited (Table 6), 10 completed the study as per protocol. One patient (P011) withdrew consent one day into the second multiple dosing phase for social reasons. Patient number P013 underwent single dosing with remacemide but was subsequently withdrawn from the trial because one of his pre-trial phenytoin levels was found to be below the target range. After dosage adjustment, the patient recommenced the study as patient P014.

Phenytoin pharmacokinetics

There were no changes in phenytoin pharmacokinetic parameters following a single dose of remacemide hydrochloride (Figure 11 and Table 6). Following 14 days' treatment, however, there was a trend towards an increase in the mean $AUC_{0\text{-}12h}$ of phenytoin by 11.5% (p=0.33), the mean Cmax by 13.7 % (p=0.32), and the mean Cmin by 22 % (p=0.12). Tmax was unchanged following both single and multi-dose remacemide hydrochloride. Comparison of mean trough phenytoin levels 5, 12, and 15 days after the start of active treatment (76.8, 95.4 and 90.6 ng/ml respectively) with those on placebo (72.1, 73.0 and 74.7 ng/ml respectively) showed a treatment associated increase which reached statistical significance (p=0.02). No patients, however, reported any symptoms suggestive of phenytoin toxicity.

Table 6

Demographic characteristics of phenytoin-treated population

Patient	Sex	Age (years)	Phenytoin dose (mg/day)	Dosage interva (hours)
P 001	M	59	450	12
P 007	M	28	350	24
P 008	M	28	300	12
P 009	M	58	400	12
P 010	M	27	300	24
P 011*	M	22	275	24
P 012	M	31	200	12
P 014**	M	54	500	12
P 015	M	26	600	12
P 016	F	41	325	12
P 017	F	24	350	12

* Did not complete study
**Was initially included as Patient number 13, withdrawn and re-entered as 014.

Table 7

Mean phenytoin pharmacokinetic parameters (SD) after single and multiple doses of remacemide hydrochloride and placebo in 10 epileptic patients

	AUC ($nmol.hr.ml^{-1}$)	Cmax ($nmol.ml^{-1}$)	Tmax (hours)	Cmin ($nmol.ml^{-1}$)
Placebo	944 (459)	98.3 (57.3)	4.7 (3.6)	74.7 (34.4)
Single dose	917 (315)	90.9 (30.2)	3.3 (3.6)	80.0 (31.4)
Multiple doses	1047 (533)	108 (55.1)	3.9 (3.6)	87.8 (44.2)

Figure 11

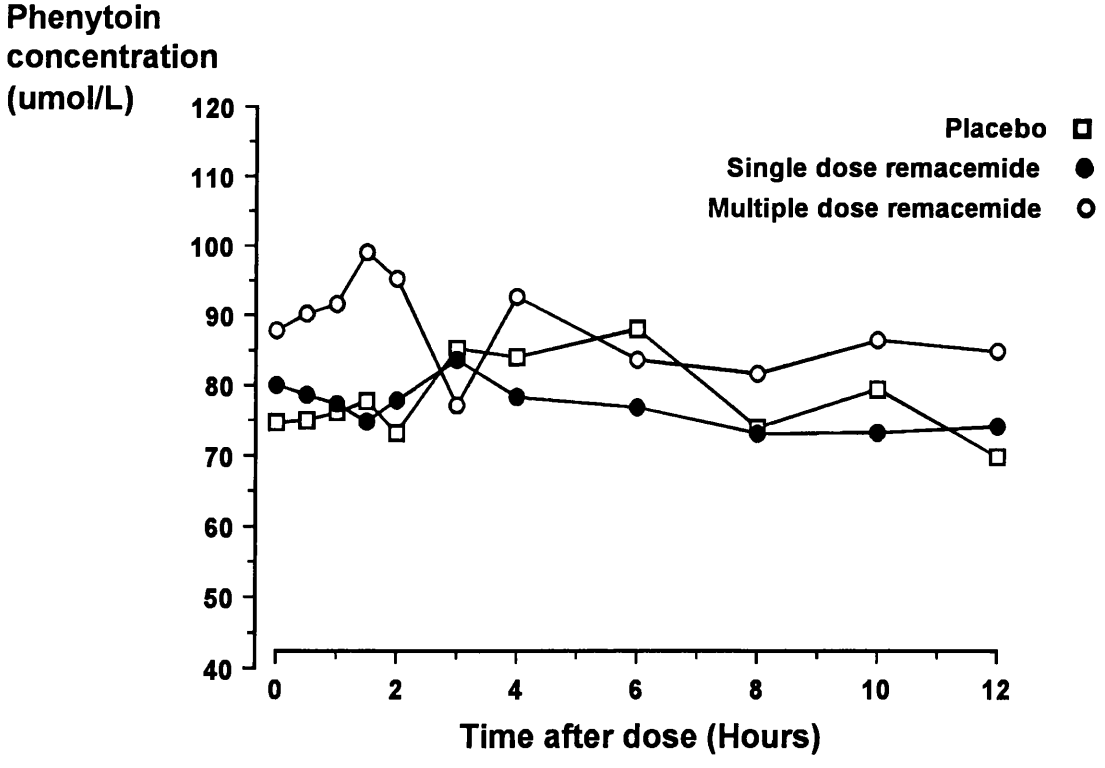


Figure 11: Mean plasma concentrations of phenytoin in 10 patients following treatment with single (300mg) or multiple (300mg BD for 14 days) dose of remacemide hydrochloride.

Remacemide and ARL12495XX pharmacokinetics

Mean pharmacokinetic parameters for remacemide hydrochloride and ARL12495XX following single and multiple dosing are shown in Table 8, and are similar to the parameters demonstrated in carbamazepine-treated individuals. As anticipated from a drug with a considerably shorter half-life than dosing interval, there was little carry-over of remacemide from dose to dose, and the steady-state profiles attained only slightly higher levels than those following the single dose. For ARL12495XX, consistent with its longer terminal half life, there was a greater carry-over during multiple dosing, and at steady-state the maximum concentrations attained were almost twice those following the single dose. With both remacemide and ARL12495 (Figure 12 and 13), there was good predictability of multiple dose profiles compared with single dose profiles based on linear superposition of the concentration data suggesting a lack of autoinduction of remacemide or ARL12495XX metabolism.

Adverse events

No major adverse events occurred, and noone required withdrawal from the study due to intolerable adverse events. Similar numbers of adverse events were reported while patients were on placebo (10 adverse events) as were reported during remacemide treatment (9 events reported). An identical number of patients (six) reported adverse events following multiple dosing with active treatment as occurred on placebo, and there were similar numbers of

Table 8

Mean pharmacokinetic parameters (SD) of remacemide hydrochloride and ARL12495XX in 10 phenytoin-treated patients following acute and chronic dosing.

	Cmax ($ng.ml^{-1}$)	Cmin ($ng.ml^{-1}$)	Tmax (minutes)	AUC * ($ng.hr.ml^{-1}$)	t1/2 (hours)
Single Dose					
Remacemide	660 (191)	NA	60 (20)	1424 (511)	3.0 (0.9)
ARL12495XX	35.5 (11.5)	NA	96 (37)	210 (70)	9.1 (1.9)
Multiple Dose					
Remacemide	666 (159)	25.1 (15.4)	63 (26)	1651 (529)	2.6 (0.3)
ARL12495XX	52.7 (18.1)	25.7 (12.2)	225 (447)	372 (128)	10.1 (4.9)

* AUC_{∞} for single dose profiles, $AUC_{0\text{-}12h}$ for multiple dose profiles
NA = not applicable

Figure 12

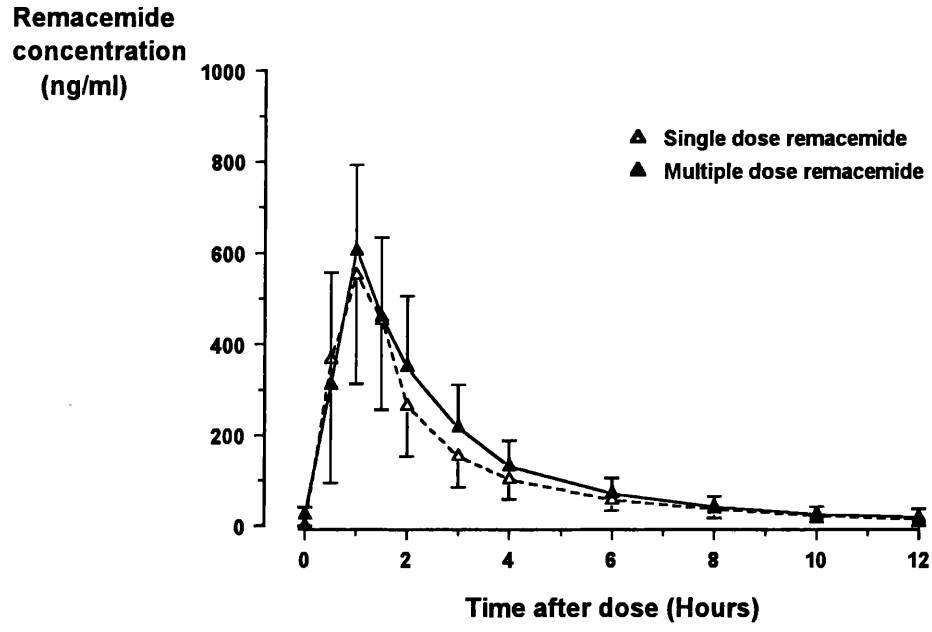


Figure 12: Mean (+/-SD) single dose and steady state plasma concentrations of remacemide in 10 patients taking phenytoin who received a single dose (300mg) or multiple doses (300mg BD for 14 days) remacemide hydrochloride.

Figure 13

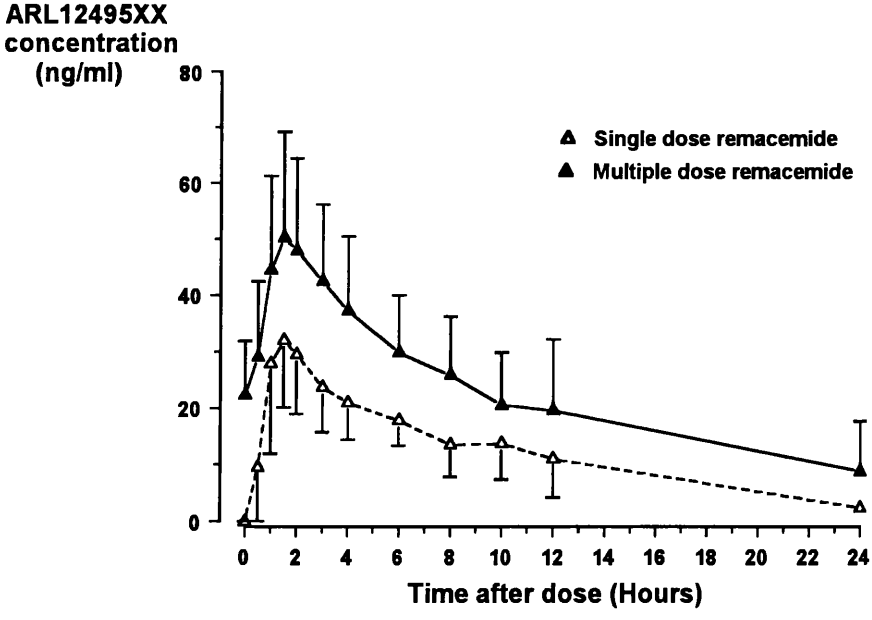


Figure 13: Mean (+/-SD) single dose and steady state plasma concentrations of ARL12495 in 10 patients taking phenytoin who received a single dose (300mg) or multiple doses (300mg BD for 14 days) remacemide hydrochloride.

central nervous system events (8 on remacemide versus 6 on placebo). No gastrointestinal symptoms were reported during this study.

Discussion

Phenytoin is a well-known inducer of the hepatic P450 mono-oxygenase enzymes (Brodie 1992). This results in marked intra- and inter-individual variations in serum concentrations of other antiepileptic drugs during polypharmacy. In addition, many other drugs have been shown to interact with phenytoin (McKee and Brodie 1995). Its clearance in man is almost exclusively by hepatic metabolic transformation, a major pathway involving the action of CYP2C9 and CYP2C19 (Levy 1995). In one study a proportion of patients on co-medication with remacemide and phenytoin had dose-related increases in trough concentrations of phenytoin, although no one required a reduction in phenytoin dose (Clark et al 1995).

Following multiple dosing with remacemide hydrochloride, there was a trend towards inhibition of phenytoin metabolism during active treatment compared with placebo which did not reach statistical significance. No patients demonstrated any clinical signs or symptoms of phenytoin toxicity.

This study confirms the effects of enzyme induction on the pharmacokinetic profile of remacemide and ARL12495XX. For both compounds, the multiple dose profile was consistent with that predicted from the single dose, indicative of linear disposition and the absence of autoinduction of metabolism. The terminal half life of remacemide was similar to that found in previous clinical studies in human volunteers, whereas that of ARL12495XX in enzyme-

induced patients was shorter than in untreated volunteers (Palmer et al 1993). In healthy volunteers, the AUC following similar dosing with remacemide hydrochloride was between two and three times higher that in patients on phenytoin in this study. Consequently, 600mg per day is unlikely to be the maximum tolerated dose of remacemide hydrochloride in patients receiving treatment with phenytoin.

The combination of phenytoin with remacemide was well tolerated, with few adverse events reported, and no patients requiring withdrawal from the study. A similar number of adverse events were reported during the active treatment and placebo phases.

There was considerable inter-patient variability in the pharmacokinetic response following the introduction of remacemide hydrochloride, suggesting differences in individual susceptibility to the phenytoin / remacemide interaction. Although vigilance should be exercised in adding remacemide hydrochloride to antiepileptic drug regimes containing phenytoin, it is unlikely that a reduction in phenytoin dosage will be required in many patients. Since the presence of phenytoin will result in lower bioavailability of remacemide and ARL12495XX, patients pre-treated with an enzyme-inducer such as phenytoin will require higher doses of remacemide hydrochloride than non-induced patients. In addition, the remacemide concentration can be expected to rise when phenytoin is withdrawn. Since remacemide and ARL12495XX exhibit predictable and linear kinetics in phenytoin patients, with no evidence of autoinduction, there should be little need for routine therapeutic monitoring of either drug in this clinical setting. The mutual interaction between phenytoin

and remacemide hydrochloride is predictable and modest, and should not present a barrier to their widespread clinical use in combination.

Valproate-treated patients

Sixteen valproate-treated patients were recruited (Table 9), each on a regime which had been stable for at least three months. All patients had at least one plasma measurement of valproate within the target range (345-695umol/L) during that period.

Patients

Of the 16 patients recruited (Table 9), 4 completed the study at the higher dose of remacemide (see protocol) with ten patients completing the trial having received 150mg for at least 9 days. Two patients withdrew from the trial due to the onset of adverse events (one at each dose level). One patient who was withdrawn during the placebo phase because of intercurrent illness, restarted the study and completed the trial at the lower dose.

Valproate pharmacokinetics

Analysis of those patients stabilised on 150mg BD remacemide shows no changes in valproate pharmacokinetic parameters following administration of single or multiple dosing of remacemide hydrochloride (Figure 14 and Table 10).

Table 9

Demographic characteristics of Valproate-treated patients treated with remacemide.

Patient	**Sex**	**Age** (years)	**Daily valproate intake over 2 divided doses** (mg)
V 002	M	38	2000
V 004	M	20	3000
V 008	F	45	1200
V 009	M	43	2000
V 010	M	24	800
V 011	M	37	1200
V 012	M	41	2500
V 022	F	24	1000
V 023	F	52	2400
V 024	M	33	3500
V 003[1]	M	54	2600
V 005[2]	M	57	1700
V 007[2]	F	19	700
V 016[2]	F	19	2000
V 001[3]	F	62	1800
V 006[4]	M	45	2000

[1]Completed high dose (300mg BD Remacemide) but results uninterpretable.
[2]Completed high dose remacemide regimen
[3]Withdrew due to adverse events at 300mg BD
[4]Withdrew due to adverse events at 150mg BD

Table 10

Mean valproate pharmacokinetic parameters (SD) after single and multiple doses of remacemide hydrochloride and placebo in 10 epileptic patients receiving 300mg final dose after multiple dosing with 150mg BD.

	AUC ($ng.hr.ml^{-1}$)	Cmax ($ng.ml^{-1}$)	Tmax (hours)	Cmin ($ng.ml^{-1}$)
Placebo	6492.9 (1857.4)	836.3 (219.5)	2.6 (1.4)	491.4 (102.9)
Single dose	6704.1 (2252.1)	775.8 (255.7)	4.5 (2.0)	533.0 (158.7)
Multiple doses	6451.2 (1348.5)	793.1 (193.7)	3.6 (2.0)	482.8 (72.6)

Figure 14

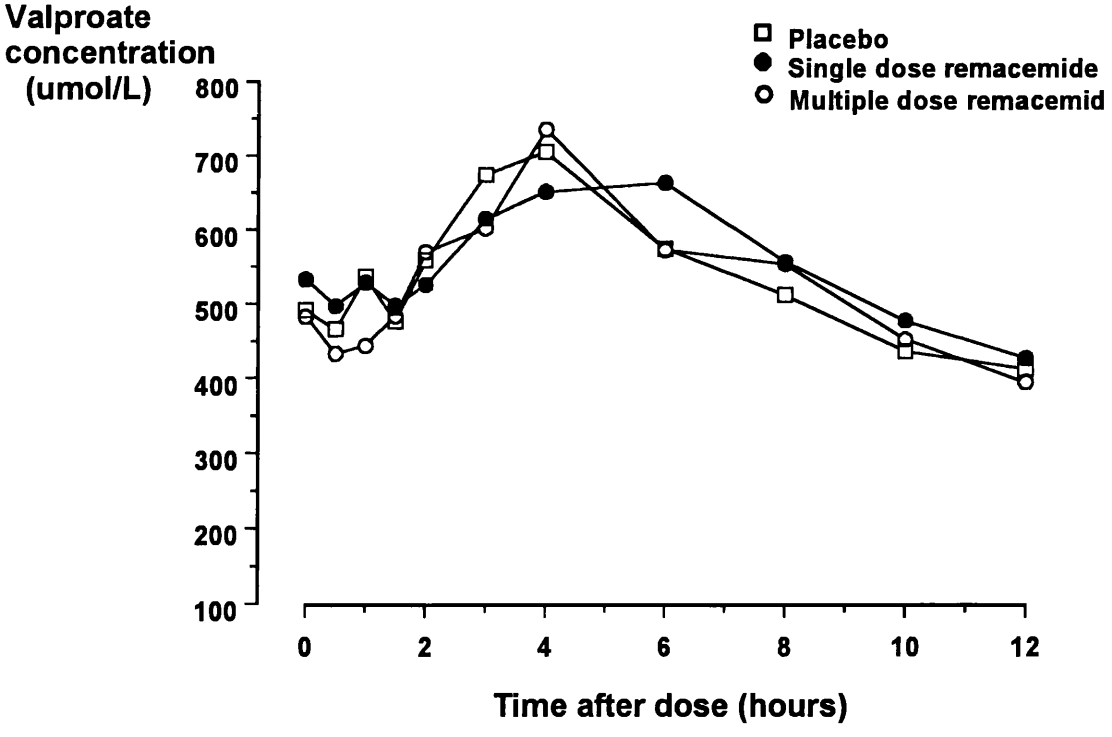


Figure 14: Mean plasma concentrations of sodium valproate in 10 patients following treatment with single (300mg) or multiple (14 days) dose of remacemide hydrochloride.

Remacemide and metabolite pharmacokinetics

Mean pharmacokinetic parameters for remacemide hydrochloride and ARL12495XX following single and multiple dosing are shown in Table 3V. After 14 days treatment, there was no significant difference in any of the pharmacokinetic parameters of remacemide or ARL12495XX (Figures 15 and 16).

Based on linear superposition of the concentration data, there was good predictability of multiple dose profiles of both remacemide and ARL12495XX compared with single dose profiles, suggesting a lack of autoinduction of remacemide or ARL12495XX metabolism.

The half life of both remacemide and ARL12495XX was lower in the 3 evaluable patients completing the multiple dose phase on 300mg BD (Table 12) compared to those intolerant of the high dose, although with small numbers involved, this did not reach statistical significance.

Adverse events

Of the nine patients started on remacemide hydrochloride 300mg BD, two were withdrawn from the study: one immediately, and one following a reduction in remacemide hydrochloride dose to 150mg BD. Two other patients had the dose reduced to 150mg BD after 4 days, and completed the trial satisfactorily at this dose level. The side effects experienced at 300mg BD, were central nervous system related (dizziness, drowsiness, headache) with one patient experiencing dyspepsia which failed to resolve on reduction of the remacemide hydrochloride dose.

Table 11

Mean pharmacokinetic parameters (SD) of remacemide hydrochloride and ARL12495XX in 10 valproate-treated patients following acute and chronic dosing.

	Cmax ($ng.ml^{-1}$)	**Cmin** ($ng.ml^{-1}$)	**Tmax** (Hours)	**AUC*** ($ng.hr.ml^{-1}$)	**t1/2** (hours)
Single Dose					
Remacemide	817 (257)	NA	1.3 (0.8)	3282 (899)	4.2 (0.7)
ARL12495XX	76.7 (25)	NA	5.2 (2.7)	1310 (7583)	15.5 (3.8)
Multiple Dose					
Remacemide	706 (380)	46.7 (25.0)	1.2 (0.3)	2644 (1347)	4.2 (0.9)
ARL12495XX	104 (41)	46.4 (14.1)	2.8 (1.2)	924 (364)	15.0 (5.6)

* AUC_{∞} for single dose profiles, $AUC_{0\text{-}12h}$ for multiple dose profiles
NA = not applicable

Table 12

Mean pharmacokinetic parameters (SD) of remacemide hydrochloride and ARL12495XX in 3 valproate-treated patients following acute and chronic dosing with 300mg BD.

	Cmax ($ng.ml^{-1}$)	**Cmin** ($ng.ml^{-1}$)	**Tmax** (Hours)	**AUC*** ($ng.hr.ml^{-1}$)	**t1/2** (hours)
Single Dose					
Remacemide	934 (408)	NA	1.5 (0.5)	3167 (748)	4.3 (2.1)
ARL12495XX	123 (45)	NA	3.5 (2.3)	1487 (286)	13.8 (5.9)
Multiple Dose					
Remacemide	803 (242)	61.8 (50.0)	1.0 (0.5)	2791 (572)	3.5 (0.5)
ARL12495XX	159 (39)	85.4 (15.5)	1.8 (0.3)	1351 (155)	10.3 (2.4)

* AUC_{∞} for single dose profiles, $AUC_{0\text{-}12h}$ for multiple dose profiles
NA = not applicable

Figure 15

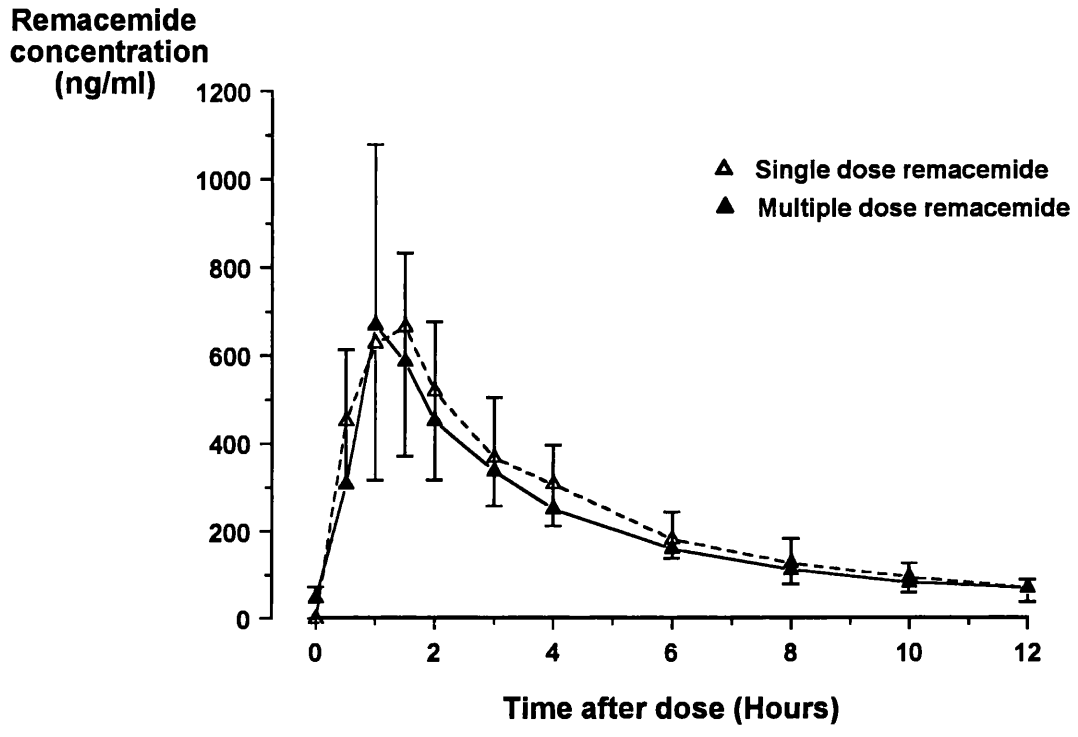


Figure 15: Mean (+/-SD) single dose and steady state plasma concentrations of remacemide in 10 patients taking sodium valproate who received a single dose (300mg) or multiple doses (300mg BD for 14 days) remacemide hydrochloride.

Figure 16

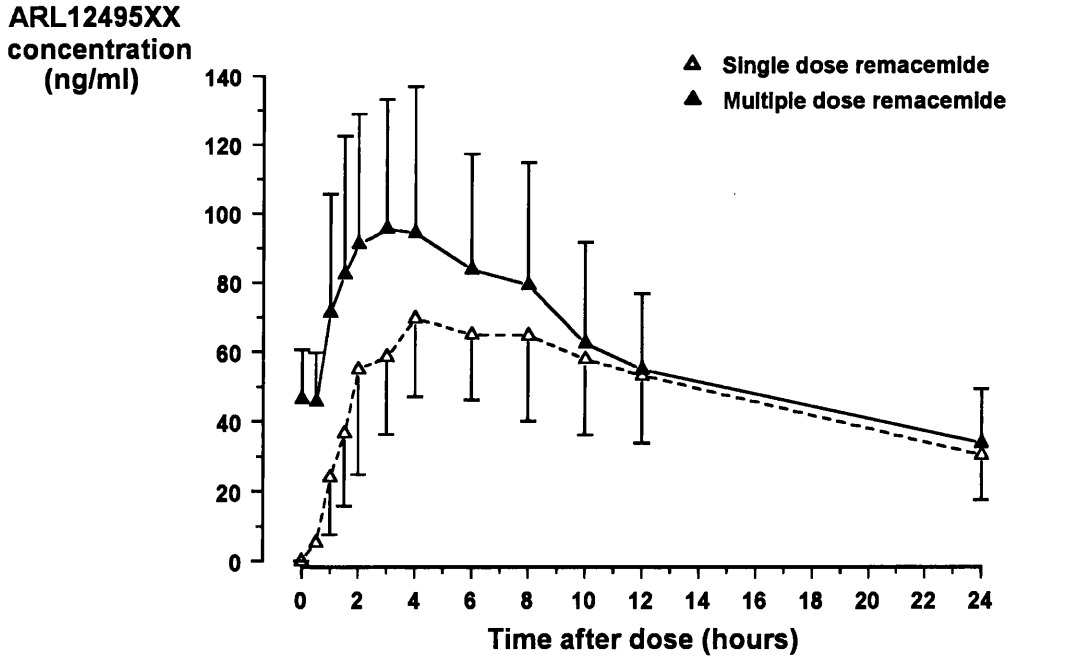


Figure 16: Mean (+/-SD) single dose and steady state plasma concentrations of ARL12495 in 10 patients taking sodium valproate who received a single dose (300mg) or multiple doses (300mg BD for 14 days) remacemide hydrochloride.

While on 150mg twice daily, no new major adverse events were reported, and as stated, only one patient was withdrawn from the study while on 150mg BD, because of an adverse event which had begun while on the higher dose (see above).

A similar number of patients on 150mg BD reported adverse events during dosing with active treatment (7 patients) as reported events while on placebo (5 patients). More adverse events were reported while patients were on remacemide treatment (21 events reported) compared to placebo (8 events reported), with more CNS side effects during active treatment (10 events) compared to the placebo treatment phase (1 event). Two gastrointestinal symptoms were reported during active treatment phase, with none occuring on placebo.

Discussion

Sodium valproate is a known hepatic enzyme inhibitor (Brodie 1992). This results in marked intra- and inter-individual variations in serum concentrations of other antiepileptic drugs during polypharmacy (McKee et al 1993). In addition, many other drugs have been shown to interact pharmacokinetically with valproate (McKee and Brodie 1995).

This interaction study used a placebo-controlled design in order to investigate the potential interaction between remacemide hydrochloride and valproate. Following multiple or single dosing with remacemide hydrochloride, there was no significant effect seen on valproate metabolism.

This study also offered the opportunity to provide a pharmacokinetic profile of remacemide and ARL12495XX in valproate-treated patients. For both compounds, the multiple dose profile was consistent with that predicted from the single dose, indicative of linear disposition and the absence of autoinduction of metabolism. The pharmacokinetic parameters of remacemide and ARL12495XX in a valproate-treated population were similar to that found in human volunteers (Palmer et al 1993). The mean terminal half life of both remacemide and ARL12495XX in valproate-treated patients after chronic dosing (4.16 and 15.03 hours respectively) are not significantly different from those in healthy volunteers (3.35 and 12.86 hours respectively) (Palmer et al 1993).

At 150mg twice daily of remacemide hydrochloride, the drug was well tolerated compared to placebo. There was a minor degree of inter-patient variability in the pharmacokinetic response following the introduction of remacemide hydrochloride suggesting differences in individual susceptibility to the interaction. Interestingly, although it did not reach statistical significance, patients who completed the study at the higher dose of remacemide displayed a shorter half life of both remacemide and ARL12495XX than those requiring dose reduction. The kinetics of remacemide and ARL12495XX, therefore, are not saturable at anticonvulsant doses. A proportion of valproate-treated patients may tolerate remacemide at the higher dose.

In this study, a higher number of adverse events were reported during the active treatment phase than during the placebo phase. Given the

pharmacokinetic data, the adverse events reported during remacemide treatment may be the result of a pharmacodynamic rather than pharmacokinetic interaction. A more complex interaction between remacemide and the various long-acting metabolites of valproate cannot, however, be excluded at this point.

Although vigilance should be exercised in adding remacemide hydrochloride to antiepileptic drug regimes containing valproate, it is likely that when started at an adequately low dose, remacemide would be well-tolerated when used with concomitant valproate.

Conclusions

As discussed, the pharmacokinetics of remacemide and its active metabolite ARL12495XX are significantly different in a population that is receiving enzyme-inducing anticonvulsant medication. There is a trend towards an increase in the mean half life of remacemide in those on valproate (4.16 hours) compared to patients pretreated with carbamazepine (3.39 hours) or phenytoin (2.59 hours) which reached statistical significance only in the latter case. The half life of ARL12495XX in valproate-treated patients, however, was significantly raised compared to patients on monotherapy with either carbamazepine (9.06 hours) or phenytoin (9.15 hours).

The effect of remacemide co-treatment on the established AEDs is variable. No significant differences were seen in phenytoin or valproate metabolism during treatment with remacemide. There was an increase in phenytoin concentrations trough concentrations which became significant after the first week.

Levels of CBZ-E are also unchanged by concomitant remacemide, but carbamazepine pharmacokinetics, are less predictable. Some patients experienced a moderate rise in peak and trough carbamazepine concentrations. It should be remembered, however, that no patients reported symptoms of carbamazepine toxicity, probably as a result of the unchanged CBZ-E levels. Further studies which are already underway, which involve potential dose altering by an unblinded observer may further elucidate the relationship between carbamazepine and remacemide.

Those patients on valproate, although lacking any significant alterations in

metabolism of remacemide and ARL124954XX are probably more susceptible to adverse events than untreated volunteers. This may be due to a pharmacodynamic interaction between valproate and remacemide, or it may conceivably be a result of a pharmacokinetic interaction between remacemide and a long-lasting metabolite of valproate.

The effects of the new AEDs on rodent whole brain biochemistry

The use of whole brain biochemistry to examine the new AEDs

As our experience with the new AEDs grows, it is becoming more apparent that their effects are more wide ranging than was previously thought. Since the metabolism of amino acids is part of a large metabolic loop, and since enzyme substrates are structurally similar, it may be naive to think that a compound will have significant effects at one point in the chain without producing significant effects elsewhere.

In this respect, investigation of enzyme and substrate effects of AEDs needs to take a wide view. Further expansion of the enzymes and amino acids assayed would be desirable in the investigation of effects on glutamine synthase. The model may allow further manipulation to assess the role of different combinations of AEDs.

The effects of vigabatrin on levels of whole brain GABA concentrations and GABA-T activity have been well documented (Schechter et al 1991). The extent of the in-vivo GAD inhibition is less well described. With tiagabine possibly working well in combination with vigabatrin (Leach and Brodie 1994), we were keen to ascertain if the biochemical effects of combining the two compounds may give a rationale for further clinical combinations. Having initially used this method to assess these two drugs in combination, we subsequently extended the scope of investigation of other AEDs to include glutamate and glutamine, both essential precursors to GABA formation. The lesson having been learnt with vigabatrin in cell culture, that drugs can often

have surprising neurophysiological effects, we decided to investigate the effects of two new AEDs, gabapentin and remacemide, on the enzymes and precursors of the GABA shunt.

Effect of ARL12495XX on whole brain biochemistry

Remacemide has been thought to have one single mode of action, but as with lamotrigine and vigabatrin (Leach and Brodie 1995), the anticonvulsant effects may be augmented by related neurochemical effects. The neurochemical properties of remacemide have already been discussed, but given our experience of other new AEDs, we would contend that it is important to assess the effect of remacemide on neuronal amino acid metabolism in-vivo. This gives us a fuller understanding of the drug and its possible usage in combination.

The desglycinyl derivative, ARL12495, is an important active metabolite of remacemide. Routinely, this is the compound that is used in in-vitro experiments of remacemide.

This experiment looks at the effect of single and multiple i.p. injections of ARL12495AA on GABA metabolism in whole brain. Metabolism of GABA was monitored by measuring it's precursors (glutamate, glutamine), total GABA levels, and the activity of the enzymes responsible for the formation (GAD) and breakdown (GABA-T) of GABA .

Study design

Acute studies

The first set of mice were given a single dose of either normal saline solution or ARL12495AA by i.p. injection. Each treatment group consisted of six mice. The dose of ARL12495AA used was 10, 25, 50, or 75mg/Kg. Six hours after

injection, the brains were removed and assayed for the parameters of GABA metabolism as described below.

Chronic Studies

The second set were given a single injection of saline or ARL12495AA at identical doses to that described above, for 5 consecutive days. Six hours after the final dose, identical procedures were undertaken.

Statistics

Calculations were carried out using the Minitab V10 software package. Results were compared using ANOVA with Dunnet correction, to compensate for multiple comparisons with control.

RESULTS

Glutamine levels

No differences were found on comparing glutamine levels of control animals, with those receiving ARL12495AA for one or more days (Figure 17).

Glutamate levels

No differences were found on comparing glutamate levels of control animals, with those receiving ARL12495AA as a single dose (Figure 18). After administration of the higher doses for 5 days, there was a trend towards a reduction in glutamate levels, although this narrowly failed to achieve statistical significance.

Figure 17

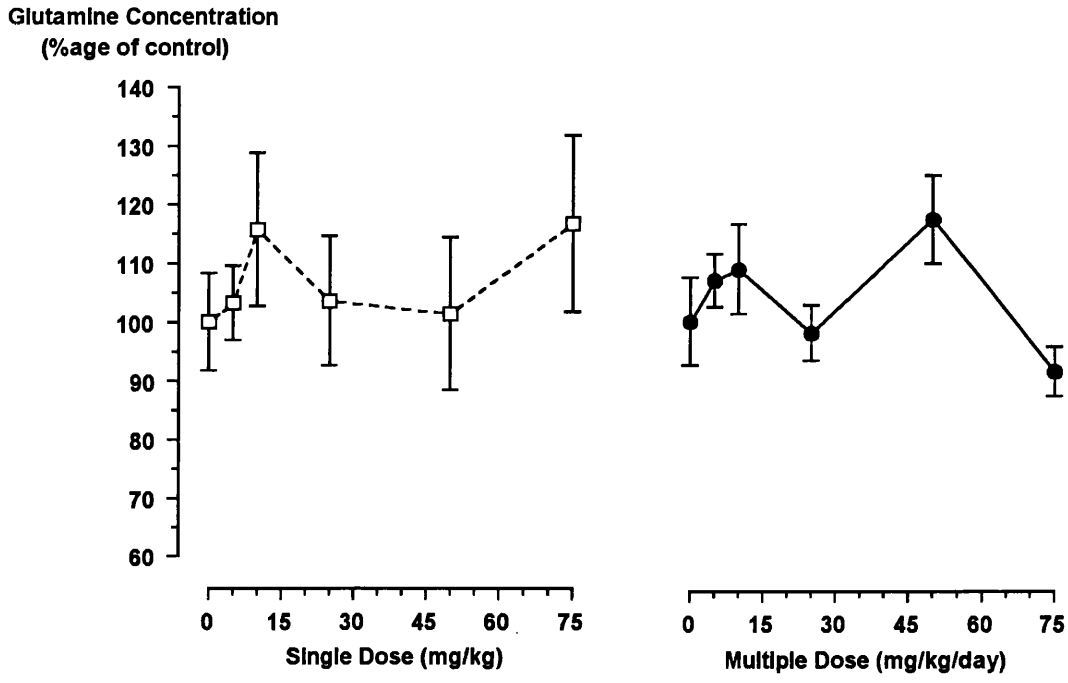


Figure 17: Mean (±SEM) glutamine concentrations in mouse whole brain after single and multiple doses of ARL12495

Figure 18

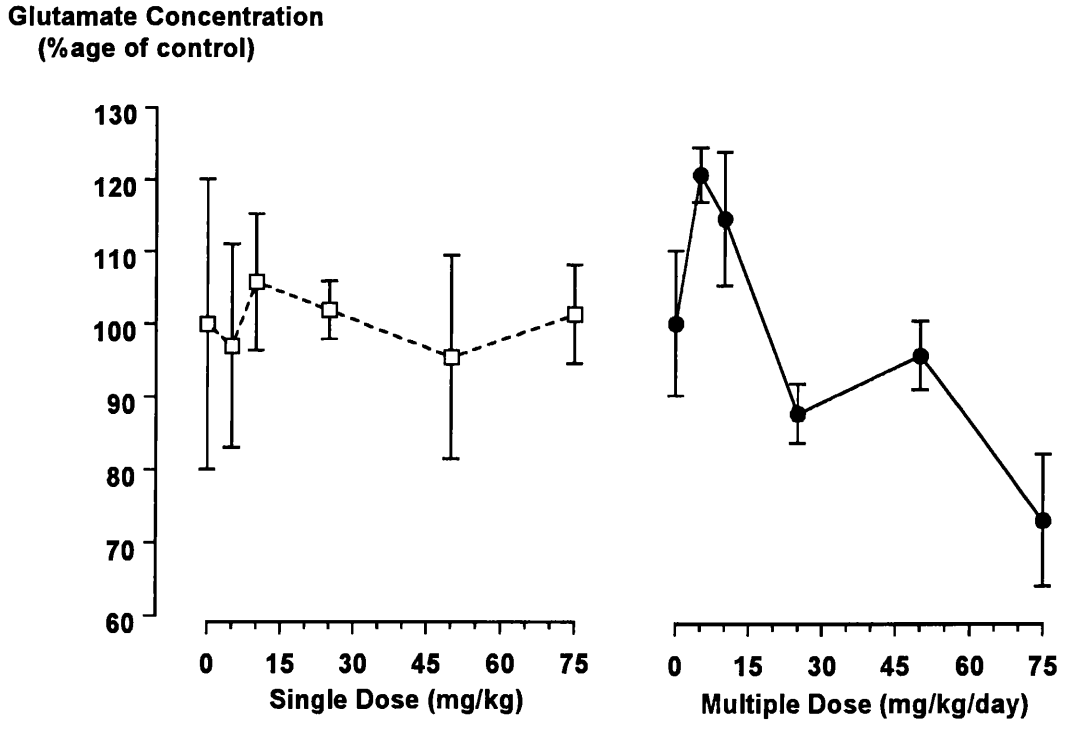


Figure 18: Mean (±SEM) glutamate concentrations in mouse whole brain after single and multiple doses of ARL12495,

GABA Levels

No differences were found on comparing GABA levels of control animals, with those receiving ARL12495AA chronically or acutely (Figure 19).

GAD activity

After a single dose there were no significant differences in GAD activity (Figure 20). When ARL12495AA had been administered for 5 days, however, at doses of 50 and 75 mg/Kg/day, a significant decrease was noted in activity of the enzyme.

GABA-T activity

Following single dose ARL12495AA, no change was noted in GABA-T activity (Figure 21). After 5 days' administration, however, at the two highest dose levels (50 and 75 mg/Kg/day), the activity of GABA-T was significantly increased.

Discussion

The enzyme effects of ARL12495AA have not been previously described for a compound that is an effective anticonvulsant. Both the decrease in GAD and the increase in GABA-T could be construed as being conducive to a decrease in GABAergic inhibition, and at first sight, this might not be the best secondary effect for a potential AED to have. We would argue, however, that these

Figure 19

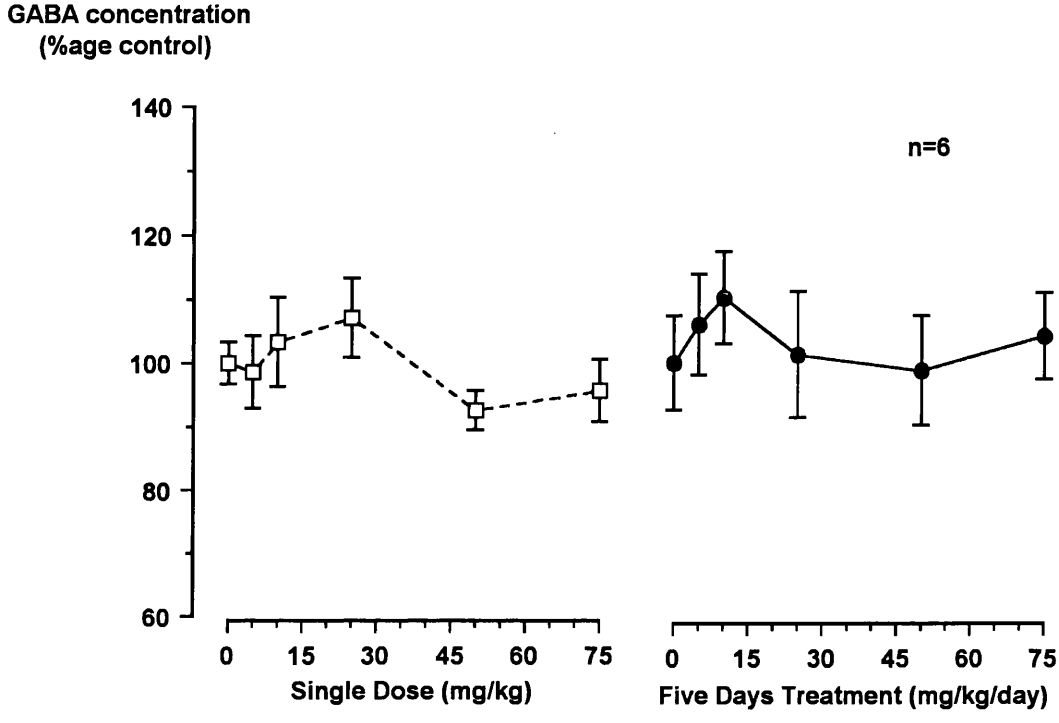


Figure 19: GABA concentrations in mouse whole brain after single and multiple doses of ARL12495

Figure 20

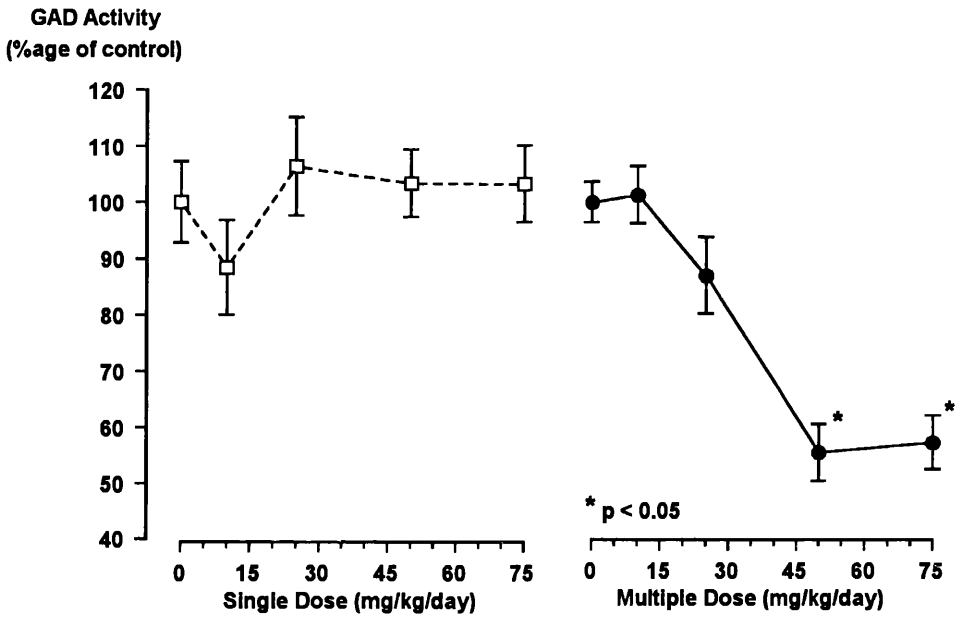


Figure 20: Activity of GAD in mouse whole brain after single and multiple doses of ARL12495

Figure 21

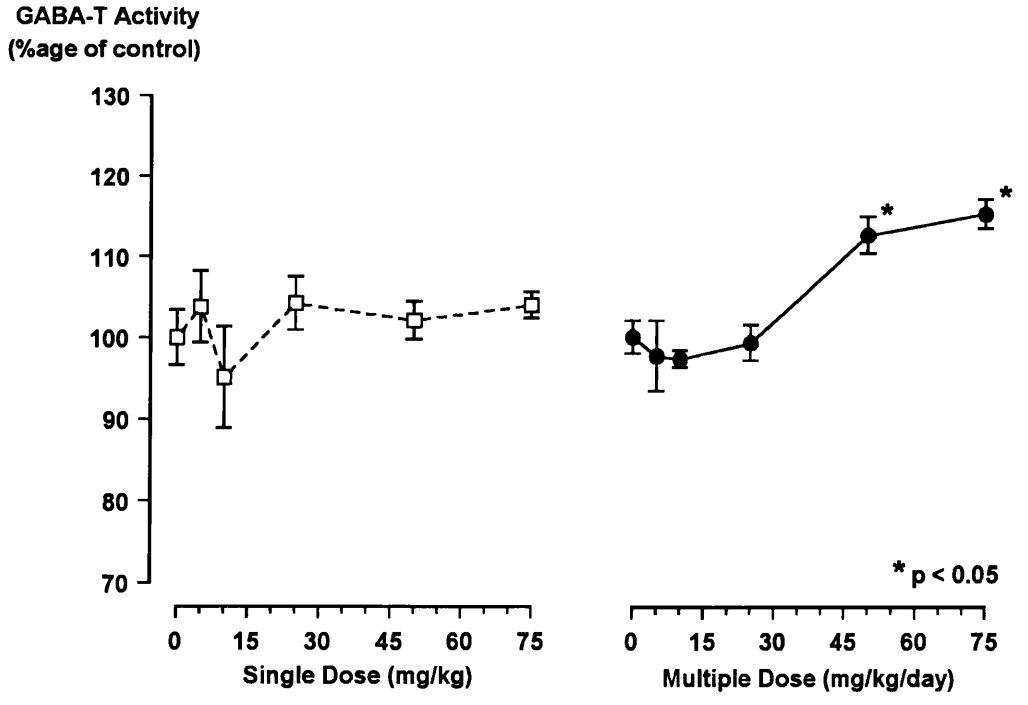


Figure 21: Activity of GABA-T in mouse whole brain after single and multiple doses of ARL12495

effects are rendered less important because the levels of whole brain GABA remain unchanged by ARL12495AA. Although measurement of whole brain GABA is not always a reliable indicator of GABAergic inhibitory tone, the lack of a fall in GABA levels is somewhat reassuring.

That the highest doses of ARL12495AA should affect GAD and GABA-T activity is perhaps not overly surprising, given it's effects on the hepatic cytochrome P450 system in-vitro (Riley et al 1995) and in-vivo (Leach et al, submitted). It should be noted that the effects on GABA-T occur only at doses approaching those maximally tolerated in rodents (Muir and Palmer 1991).

The diminished GAD activity at higher dose may be related to the decrease in concentrations of it's substrate, glutamate. This decrease in whole brain glutamate, though not statistically significant, is of some interest. Glutamate is a well recognised excitotoxin (Meldrum 1990). This neurotoxicity is mediated mainly by it's actions at the NMDA receptor (Choi et al 1991). Activation of this glutamate receptor predisposes to cationic influx which can cause cell death, and ARL12495XX has already been shown to inhibit *release* of glutamate (Srinivasan et al 1995). If further work can confirm that remacemide and its metabolites have effects not only on glutamate receptors and glutamate release, but also on glutamate levels, then it may form a rational basis for the use of the drug in other areas where excitotoxins are thought to be of importance, such as ischaemia, Parkinson's disease, and motor neurone disease.

Conclusion

Like other novel anticonvulsants, remacemide is now being shown to have wider neurochemical effects than was first thought. The nature of the effects on GABA-T and GAD were unexpected, although their significance may be tempered by the lack of an effect on total GABA levels.

Whether the enzyme changes in mice following a short duration of treatment are of any relevance to a human population treated for longer periods with relatively lower doses is, at the moment, unclear.

Effects of gabapentin on whole brain biochemistry

This paper looks at the effect on GABA metabolism of different doses of gabapentin on rodent brain following single and multiple dosing. GABA metabolism was monitored by measuring it's precursors (glutamate, glutamine), total GABA levels, and the activities of GAD and the enzyme responsible for GABA breakdown, GABA-transaminase (GABA-T).

Study design

Acute studies

One set of mice was given a single dose of either normal saline solution or gabapentin by i.p. injection. Each treatment group consisted of six mice. The dose of gabapentin administered was 5, 10, 25, 50, or 75mg/Kg. Four hours after injection, brains were removed and assays carried out for GABA, glutamate, glutamine, and the activities of GABA-T and GAD.

Chronic Studies

A second set was given twice daily i.p. injections of gabapentin, to a daily total as described above, for 8 consecutive days. Four hours after the final dose, identical procedures were undertaken.

Statistics

As with the previous study, calculations were carried out using the Minitab V10 software package. Results were compared using ANOVA with Dunnet correction, to compensate for multiple comparisons with control.

RESULTS

Glutamine levels

No differences were found on comparing glutamine levels of control animals following chronic administration (Figure 22). Acute administration showed a significant increase in glutamine levels after injection of 50mg/Kg, with no trends seen at other doses. This would cast doubt upon the importance of this single result.

Glutamate levels

Single dose of gabapentin had no effect on glutamate concentrations at any dose. After seven days, however, there was a clear trend towards reduction in glutamate concentrations, the reduction reaching statistical significance at 25mg/Kg (Figure 23).

GABA Levels

No significant changes were seen in GABA concentrations after either single or multiple dosing (Figure 24).

GAD activity

Measurement of GAD activity showed wide variability throughout all groups. There were no trends visible after single or multiple dosing (Figure 25).

GABA-T activity

Acute administration of gabapentin caused no statistically significant increases in GABA-T activity (Figure 26). After seven days, there was a clear trend towards reduction in GABA-T activity, which became significant at doses of 10mg/Kg and above (with the exception of 25mg/Kg, which narrowly failed to achieve statistical significance).

Figure 22

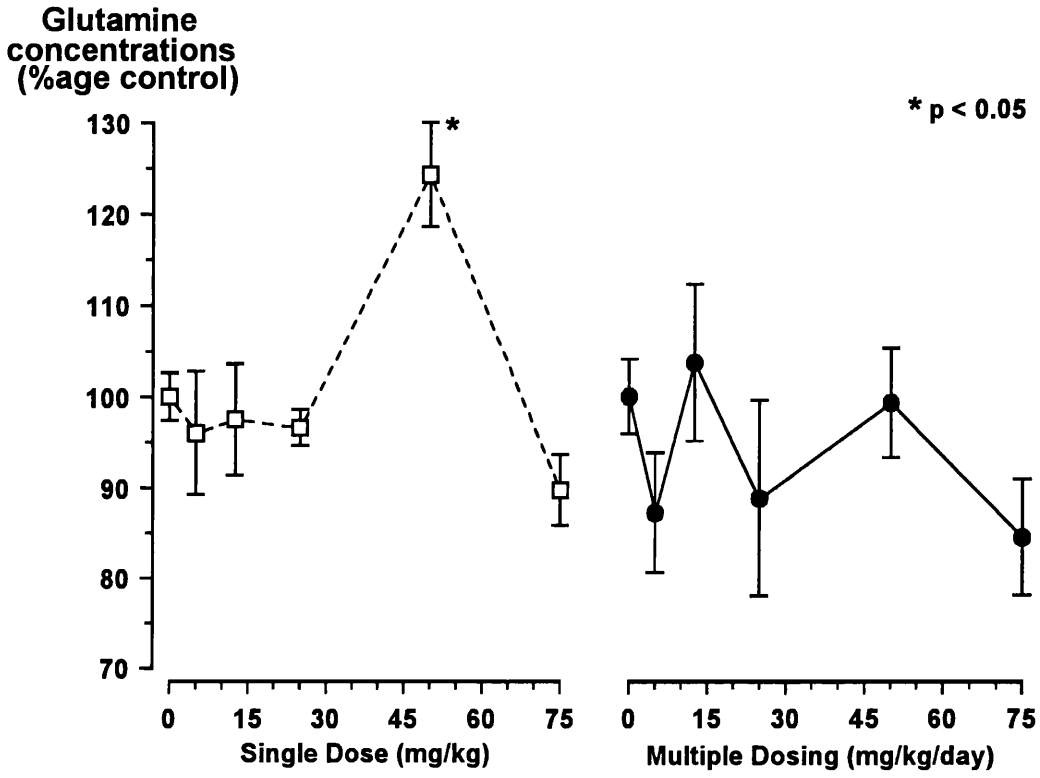


Figure 22: Mean (±SEM) glutamine concentrations in mouse whole brain following single or multiple doses of gabapentin.

Figure 23

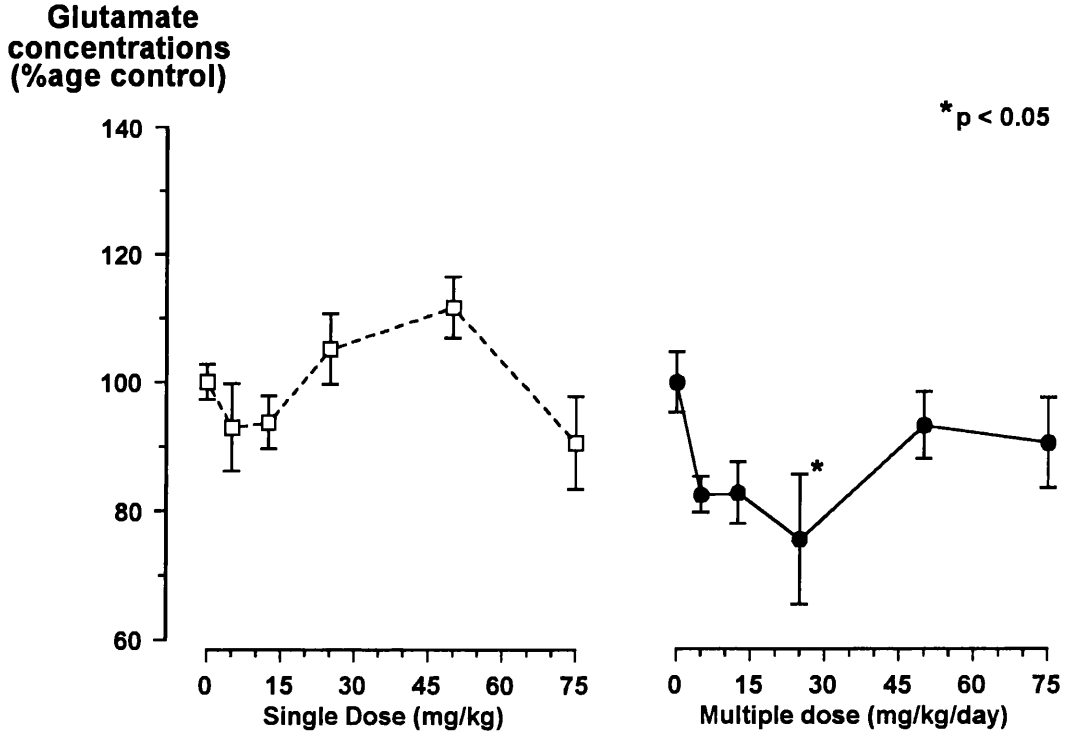


Figure 23: Mean (±SEM) glutamate concentrations in mouse whole brain following single or multiple doses of gabapentin.

Figure 24

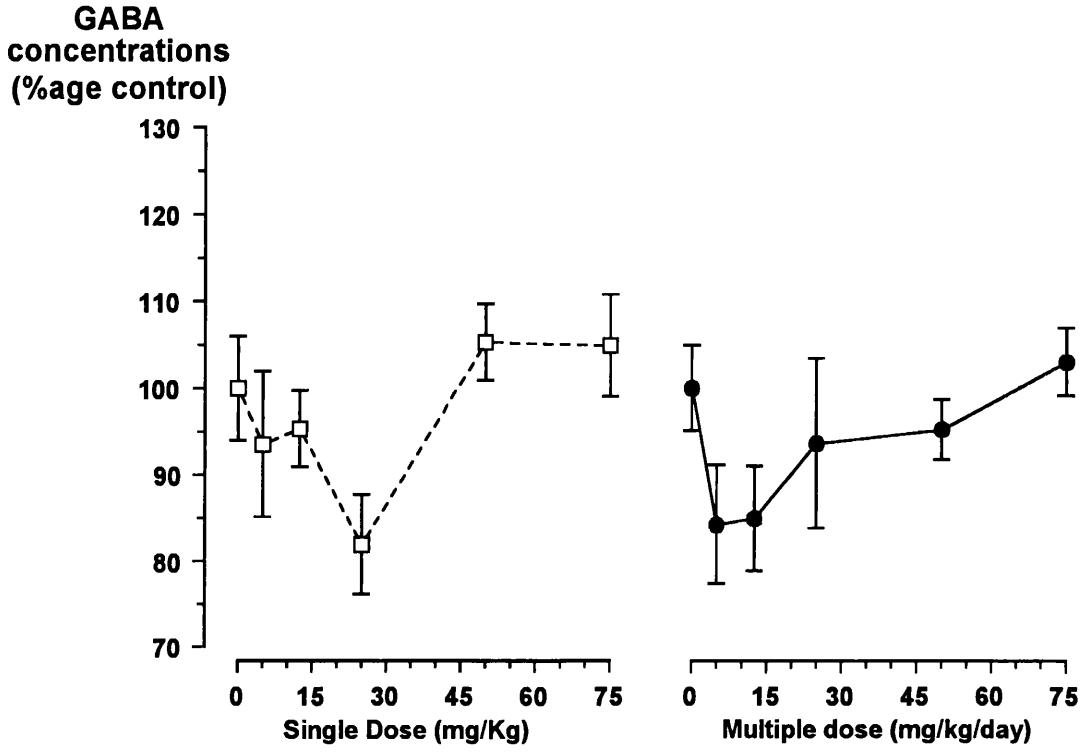


Figure 24: Mean (±SEM) GABA concentrations in mouse whole brain following single or multiple doses of gabapentin.

Figure 25

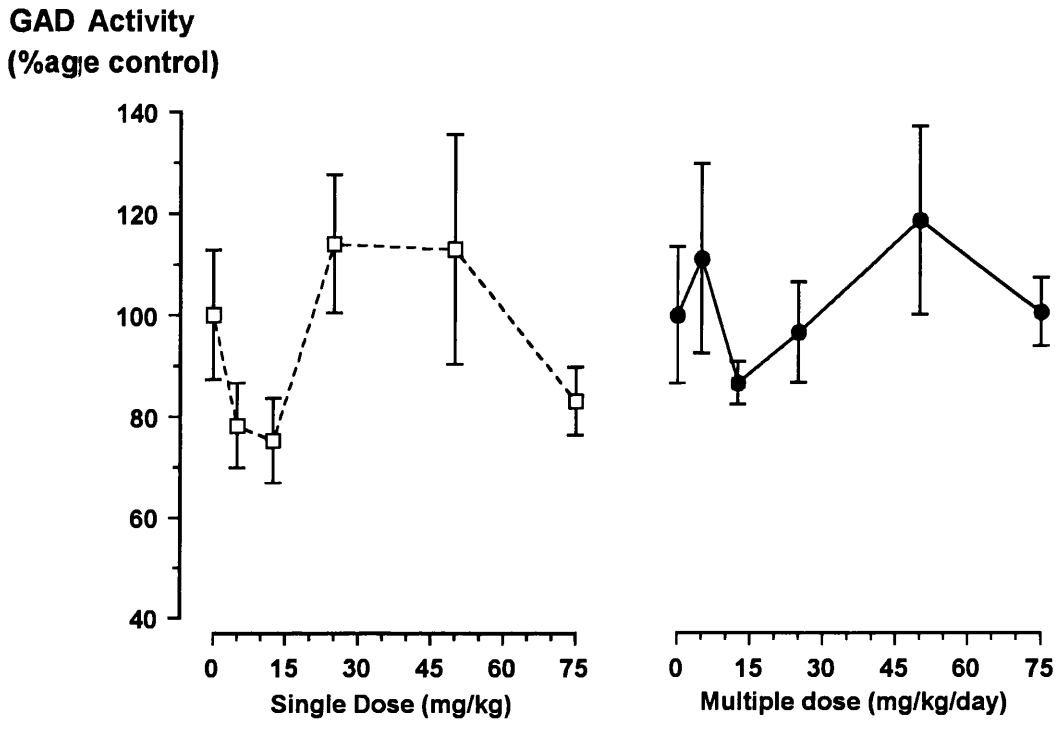


Figure 25: Mean (±SEM) GAD activity in mouse whole brain following single or multiple doses of gabapentin.

Figure 26

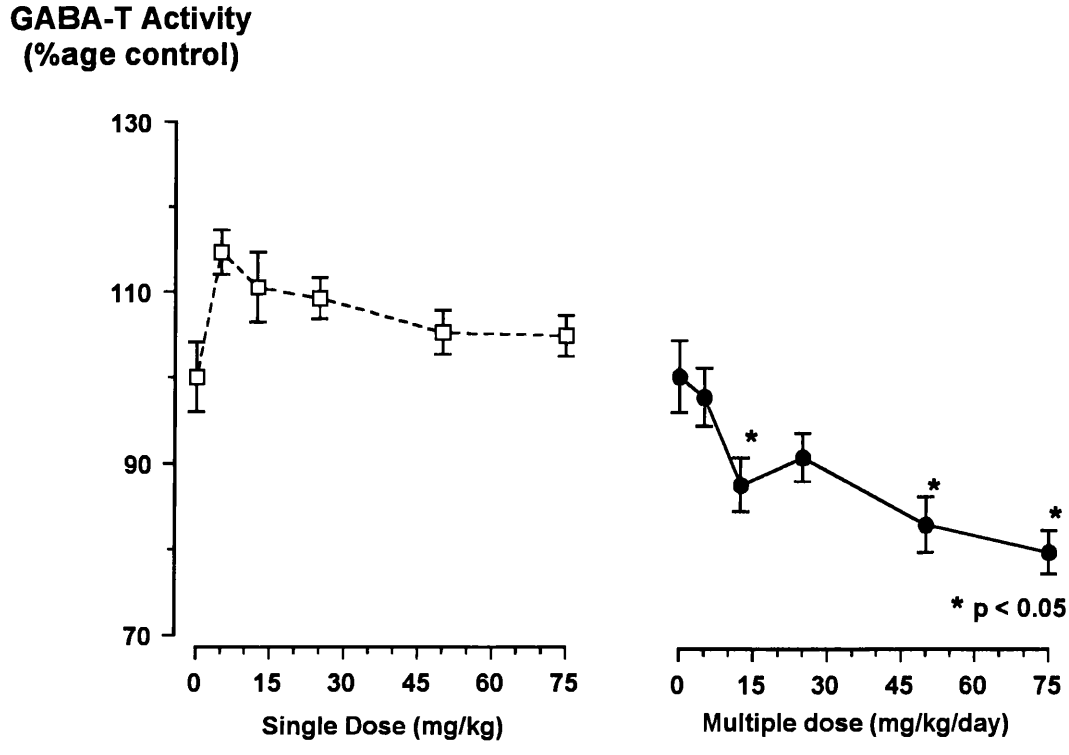


Figure 26: Mean (±SEM) GABA-T activity in mouse whole brain following single or multiple doses of gabapentin.

Discussion

Gabapentin is an efficacious and well tolerated anticonvulsant (*Chapter 2 - The new AEDs*) which has been widely used as add-on treatment for refractory epilepsy. Despite widespread searches, the exact mode of action remains unclear (Taylor 1995).

Previous in-vivo studies (Ben Menachem et al 1992) have looked at the effects of single dose gabapentin on CSF amino acids in humans, showing only that by 72 hours post-dose, the CSF levels of homovanillic acid and 5-Hydroxyindoleacetic acid were increased. The significance of these changes is unclear. Using MRI spectroscopy, gabapentin is said to increase GABA concentrations in human brain (Petroff et al, 1996). This contrasts with our findings.

We know that gabapentin does not act as a direct GABA agonist, does not bind to GABA receptors (Bartosyck et al 1986), and in fact, appears to have it's own specific binding sites as shown on autoradiography (Hill et al 1993). This may be related to a subunit of the calcium channel (Warner Lambert - Unpublished Data).

Gabapentin has been associated with various actions on the GABAergic inhibitory system. As previously discussed (Goldlust et al 1995) gabapentin was known to inhibit both GABA-T and Branched Chain Amino Acid Transaminase (BCAA-T), while Kocsis and Honmou (1994) showed that gabapentin increased non-synaptic GABA release. Induction of GAD, the enzyme responsible for production of GABA and the breakdown of glutamate, has also been thought to be of potential importance as a mode of action

(Silverman et al 1991, Taylor et al 1993, Loscher et al 1991). We found no evidence of GAD induction, and can only surmise that the aminooxyacetic acid-induced GABA accumulation demonstrated in the presence of gabapentin by Silverman and colleagues (1991) was due to an additional degree of GABA-T inhibition.

It is widely agreed that gabapentin has the potential to have many effects on GABA turnover, and our study is one attempt to further elucidate their relative importance. If we can understand how gabapentin alters the GABA shunt, then its clinical use alongside other AEDs may be better directed.

Significant inhibition of GABA-T by gabapentin may suggest that it may usefully be combined with other GABA-T inhibitors. At higher doses, vigabatrin causes an inhibition of both GAD and GABA-T activity (Leach - paper submitted). This may account for the plateau of anticonvulsant efficacy that has been shown in some trials (McKee et al 1993), and may also account for the increase in adverse events at higher doses (Grant and Heel 1992). Combination of gabapentin with vigabatrin may allow for optimal inhibition of GABA-T with less GAD inhibition, potentially improving anticonvulsant effect.

The trend towards decrease in glutamate concentrations on treatment with gabapentin may be linked to its effect on glutamate dehydrogenase discussed in earlier chapters. This decrease in glutamate levels is a desirable effect in terms of anticonvulsant and neuroprotective activity, and further work is needed to elucidate further the clinical importance, if any, of this effect.

These other associated actions of gabapentin on GABA metabolism are of great interest, and may imply that gabapentin has other wider ranging effects

on the production and breakdown of amino acids in the brain.

Conclusion

Gabapentin is an important new AED, whose mode of action is not yet fully understood. This study looks at the effect of gabapentin on the key enzymes and substrates of amino acid metabolism which are involved in the metabolism of the main inhibitory neurotransmitter GABA.

Although no changes were seen in whole brain levels of GABA during this work, gabapentin was shown to have important effects on GABA-T activity. There were significant changes in levels of glutamate after chronic dosing with gabapentin, which may be of importance in helping to lessen the negative effects of chronic refractory epilepsy.

The biochemical actions of gabapentin may allow for useful combination with other proven AEDs, particularly vigabatrin. Further clinical and laboratory studies are required to investigate the potential for use of gabapentin as part of a rational polypharmacy plan for chronic epilepsy.

Effects of tiagabine and vigabatrin on cortical biochemistry following multiple doses

Both tiagabine and vigabatrin are known to augment GABAergic inhibition. As is discussed in previous sections, there may be some additive effect on GABA uptake inhibition when both drugs are used together. During one of tiagabine's efficacy trials, we and other investigators felt that those already receiving vigabatrin tended to have a greater reduction in seizure frequency than other patients once tiagabine was added to their regime. This prompted a successful addition of sub-therapeutic doses of vigabatrin in two patients who had partially responded to tiagabine (Leach and Brodie 1994).

This experiment examines the effect on GABA metabolism of different combinations of tiagabine and vigabatrin in mice. Three parameters of GABA metabolism monitored were assayed: total GABA levels, GABA-T activity and GAD activity.

Study design

A first set of mice were split into four groups, each receiving once-daily intra-peritoneal injection with low dose vigabatrin (10 mg/Kg), low dose tiagabine (0.4mg/Kg), a combination of both drugs, or saline. Each treatment group consisted of six mice. Two further sets of mice were again subdivided into four groups and treated with similar treatment regimes at medium dose (vigabatrin 50mg/Kg, tiagabine 2mg/Kg), or high dose (vigabatrin 250mg/Kg, tiagabine 10mg/Kg). After eight days treatment the cerebral cortices were removed and

assayed for both enzymes and GABA itself.

Statistics

Calculations were carried out using the Minitab V10 software package. Results were compared using ANOVA with Dunnet correction, to compensate for multiple comparisons with control.

RESULTS

Low Dose Study

Vigabatrin and tiagabine were without effect on cortical GABA concentrations when used alone. Combination of the two at low doses however significantly increased GABA levels compared to control, although not on comparison with vigabatrin alone (Fig WC1). All other low dose treatments were without effect on the activities of GABA-T (Fig WC3) and GAD (Fig WC2).

Medium Dose Study

Both vigabatrin and combination significantly increased cortical GABA concentrations (Figure 27) compared to control, while tiagabine alone at medium dose had no effect. Vigabatrin significantly reduced GABA-T activity compared to control (Figure 28). Interestingly tiagabine caused an increase in GABA-T activity, while the combination treatment had no significant effect on

Figure 27

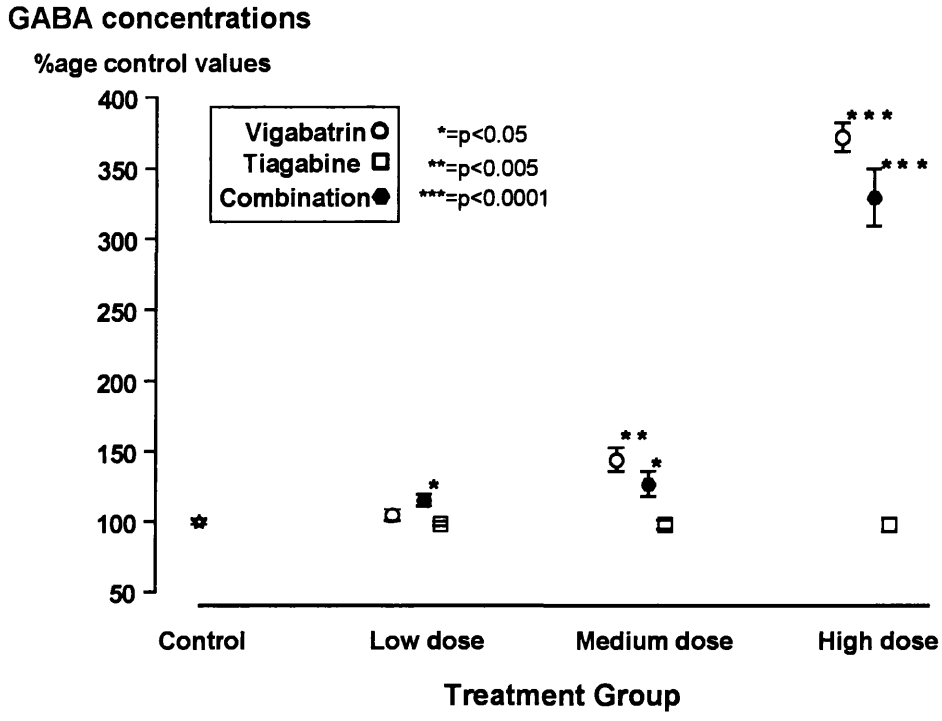


Figure 27: Mean (+/-SEM) GABA levels in mouse brain following treatment with different doses of vigabatrin, tiagabine, and a combination for 5 days.

Figure 28

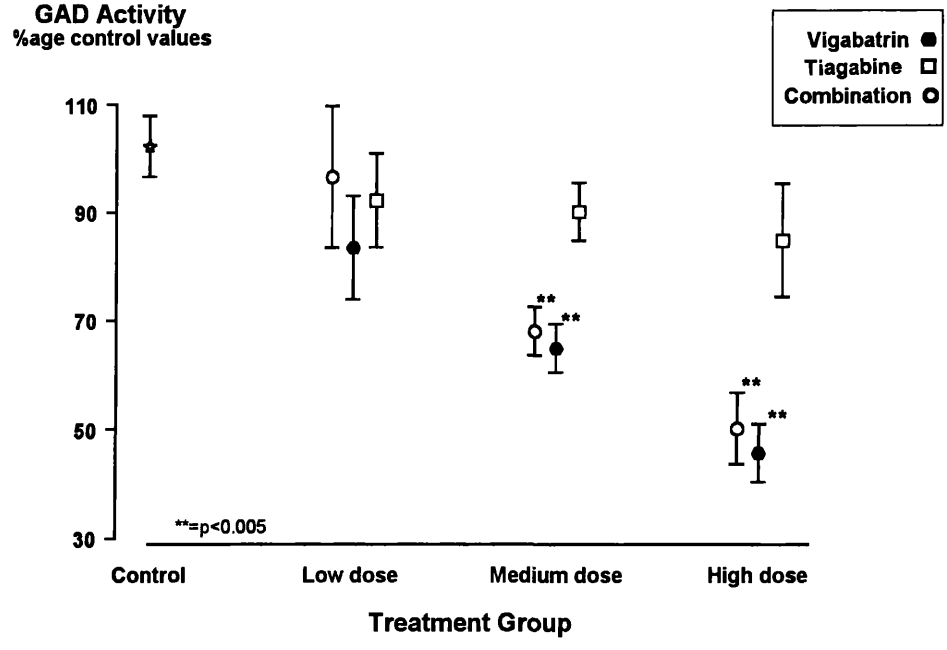


Figure 28: Mean (+/-SEM) GAD Activity in mouse brain following treatment with vigabatrin, tiagabine or a combination of the two for five days.

Figure 29

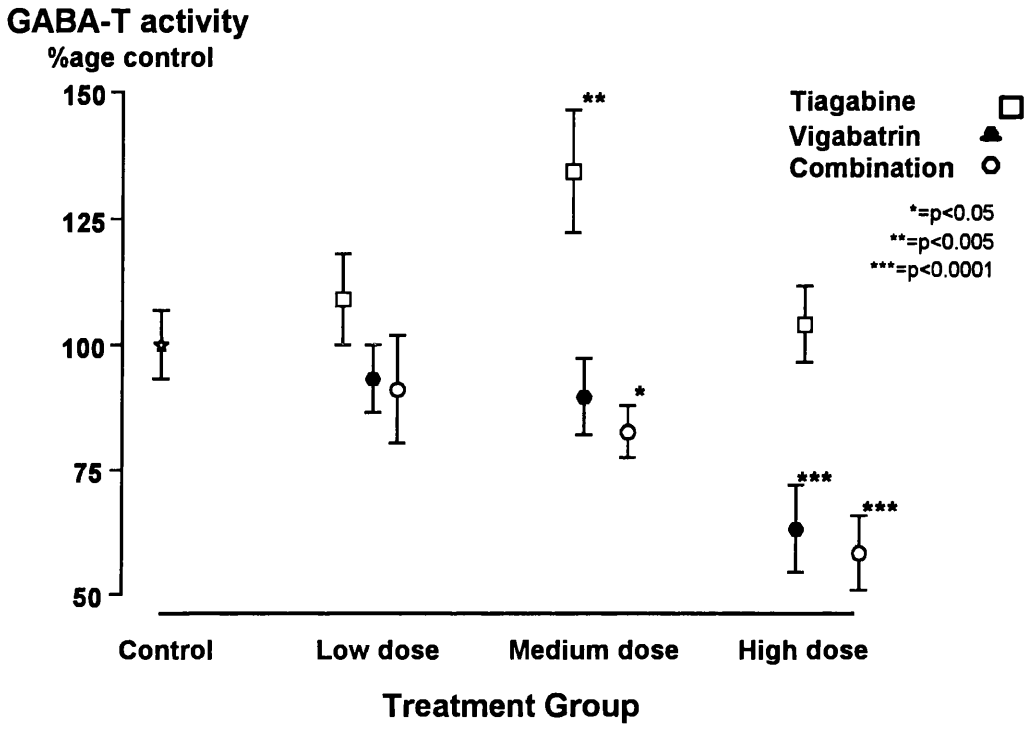


Figure 29: Mean (+/-SEM) GABA-T activity in mouse brain after treatment with various doses of vigabatrin, tiagabine or a combination for 5 days.

GABA-T activity. GAD activity was significantly reduced (Figure 28) by both vigabatrin and combination therapy at medium dose, while tiagabine alone had no effect.

High Dose Study

High dose vigabatrin and high dose combination significantly increased cortical GABA concentrations (Figure 27) and significantly reduced activity of both GABA-T (Figure 29) and GAD (Figure 28). Tiagabine at high dose was without effect on any of the parameters.

Discussion

If rational polypharmacy is to become the norm, the combined use of two GABAergic anticonvulsants would seem a reasonable first step. We have used this animal model in an attempt to delineate the biochemical effects of vigabatrin and tiagabine when used alone and in combination. Doses for this study were defined as high, medium, or low on the basis of response to chronic dosing in laboratory seizure models. Medium doses were those which were found to be optimally therapeutic, while the high and low doses were increased or decreased respectively by a factor of five. Low doses were previously shown to be subtherapeutic in our laboratories against MES and PTZ-induced seizures in mice.

Despite our earlier beliefs, vigabatrin probably has a number of effects on GABAergic function, while tiagabine is currently believed to be more specific. The finding that both may act in inhibiting GABA uptake (Leach et al 1996 -

submitted) may explain the previously noted clinical additive effect (Leach and Brodie 1994).

That low dose combination had no effect on enzyme activity, but that it increased GABA concentrations compared to control is a curious phenomenon. The observation may suggest some interaction between vigabatrin and tiagabine at low dose which underpins the additive effect seen clinically. Tiagabine has been shown to preferentially block the GAT-1 GABA uptake mechanism (Borden et al 1994), and it is not known which uptake mechanism, if any, is responsible for vigabatrin uptake. If selective GAT-1 blockade led to an upregulation of other GABA transport mechanisms, then this could theoretically enhance the intracellular uptake of vigabatrin, rendering lower doses of vigabatrin efficacious.

Medium dose vigabatrin increased GABA concentrations and decreased activity of GABA-T and GAD. The decrease in GAD activity was thought to be a result of feedback by increased levels of GABA, although this has not been thought to be a factor in mammalian brain (Horton 1989). Some authors, in contrast have suggested that vigabatrin has a direct inhibitory effect on GAD (Jung et al, 1979). It has been postulated that the effect on GAD may contribute to the development of tolerance (Neal and Shah 1990) or the limitation of effect of higher doses (McKee et al 1994) of vigabatrin. Medium dose tiagabine failed to significantly affect GABA concentrations or GAD activity, but caused a significant increase in GABA-T activity when compared to control.

At high dose, vigabatrin had a more pronounced inhibition of GABA-T, GAD,

and increase in GABA than happened at other doses. High dose combination did not differ from the actions of vigabatrin alone, suggesting that no interactions occur between the two drugs at this high dose level.

Conclusion

Tiagabine and vigabatrin are both effective novel anticonvulsants, which were thought to have different, single actions on the GABAergic inhibitory system. Both have anticonvulsant efficacy when used with other anticonvulsant agents, and some clinical experience has suggested that there is a particular benefit in the combination of both compounds. Combining these drugs at low dose may avoid the need for high doses to be used, and so decrease the incidence of adverse effects.

The effects of vigabatrin and tiagabine on GABA uptake in primary cultures of rodent astrocytes.

The role of glial cell and neuronal culture in the elucidation of basic mechanisms

Culture of glial cells has been essential in helping to elucidate their normal physiological function. As our techniques have been refined, it has now become apparent that the two morphological types of astrocytes Type I and II have differing biochemical functions.

As a result of the techniques used, most astrocyte cultures have usually consisted almost exclusively of Type II astrocytes. Since culture techniques can now be carried out to culture either type preferentially (Juurlink and Hertz 1992), there are greater possibilities for further basic research into drug mechanisms. The role of tiagabine or vigabatrin in altering GABA uptake in each astrocyte type may be important. Could gabapentin affect GABA production selectively in one or other astrocyte type?

Neuronal culture is also carried out at our Unit. Preliminary studies have confirmed that, like the nipecotic acid derivatives, vigabatrin affects GABA uptake into both neurones and astrocytes. Neuronal culture is a more challenging procedure in many respects: harvest being required from rodent fetal material, cell yield being lower, and the culture failure rate being higher. Despite these difficulties, neuronal culture may be as relevant as astrocytes in assessing the basic mechanisms of the new AEDs.

The effect of vigabatrin and tiagabine on GABA uptake in cultured astrocytes.

Cell cultures are a well established way of examining the in-vitro biochemistry of neurones and astrocytes. Although no-one would argue that any conclusions should be directly extrapolated to human populations, these techniques can provide useful clues to the mode of action of new drugs. As previously discussed, earlier work from our laboratories suggested that GABA-uptake in cultured cells may be affected by vigabatrin (Sills et al, 1993).

Aims

Our primary objective was to confirm, and if possible quantify the effect of vigabatrin and tiagabine on the rate of GABA uptake by cultured astrocytes. The doses at which this process is affected, the rapidity of onset and the duration of action will also be examined. The possibility that vigabatrin and tiagabine could act synergistically in the inhibition of GABA uptake is also to be investigated.

Methods

These are described in detail in an earlier chapter (Materials and Methods). The drugs and their doses used are as follows:

1) Tiagabine dose-ranging

Tiagabine at concentrations of 10, 50, 100, 200, 300, 400, or 500 ηmol for four hours.

2) Vigabatrin dose-ranging

Vigabatrin at concentrations of 1, 10, 50, 100, 250, 500 μmolar for four hours.

3) Tiagabine time-ranging

200ηmol tiagabine for between 0.5 hours and 24 hours.

4) Vigabatrin time ranging

100μ moles vigabatrin solution for times varying from 1 to 24 hours.

5) Combination regimes

Combination of optimal combinations of vigabatrin and tiagabine, with GABA uptake measured 4 hours after exposure.

Statistical methods

The experiment was carried out over several batches. Combination of the groups was carried out once all results were expressed as a proportion of mean control values ± the standard error of the mean. Analysis of variance was done, where appropriate using Dunnet (comparison against control data) or Tukey's corrections (comparison of variance of each group with all others).

Results

1) Tiagabine dose ranging study

Following four hour exposure of astrocytes to tiagabine at concentrations between 100 and 300ηmol, there was a significant reduction in GABA uptake into primary cultures of rat cortical astrocytes (Figure 30). Doses outwith this range were without effect on GABA uptake.

2) Vigabatrin dose-ranging study

Vigabatrin significantly reduced GABA uptake into astrocytes at concentrations between 100 and 250μmol after four hours' exposure (Figure 31). All other doses of vigabatrin were without effect.

Figure 30

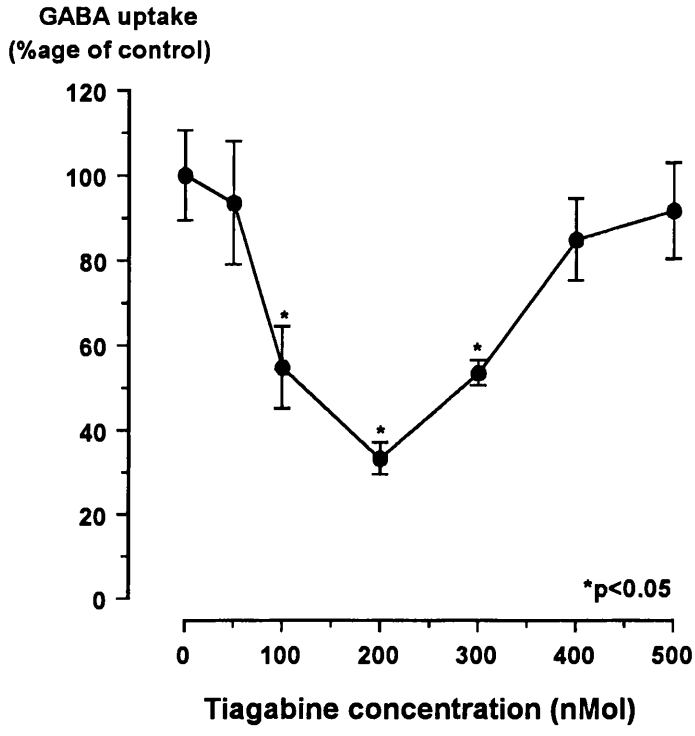


Figure 30: Mean GABA uptake (±SEM) in primary cultures of rat astrocytes in response to tiagabine for four hours.

Figure 31:

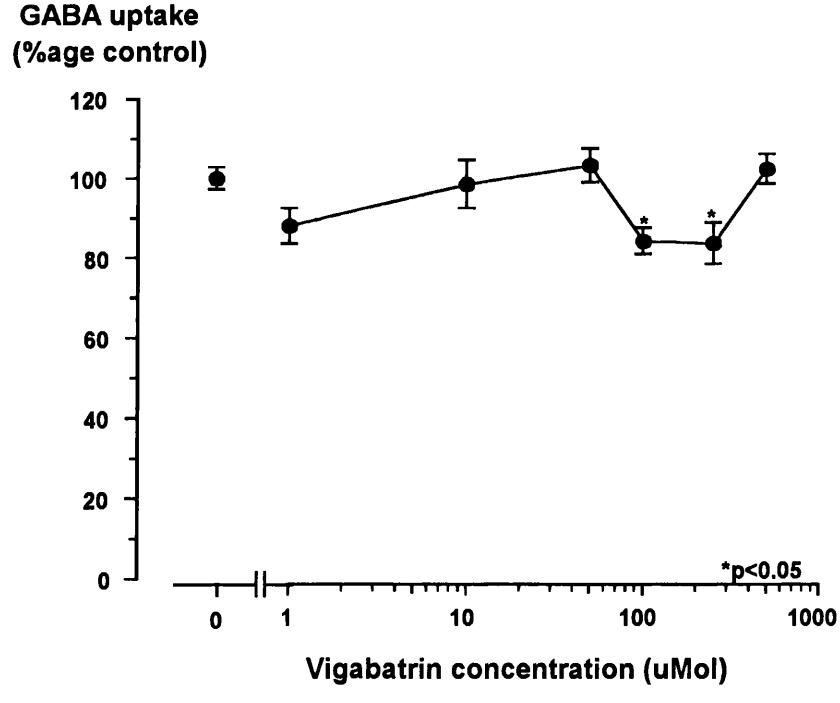


Figure 31: Mean GABA uptake (±SEM) in primary cultures of rat astrocytes in response to tiagabine for four hours.

3) Tiagabine time ranging

Tiagabine (200ηmol) significantly reduced GABA uptake four, eight, and twenty four hours post exposure (Figure 32). No effect was seen at earlier time points.

4) Vigabatrin time ranging study

Vigabatrin (100μmol) had no effect on GABA uptake at any of the time points tested (Figure 33).

5) Combination testing

Tiagabine (200ηmol), vigabatrin (100μmol) and combination treatments significantly reduced GABA into primary cultures of rat astrocytes compared to control (Figure 34). Analysis showed that combination of the two compounds was more effective than either of the drugs used singly.

Figure 32

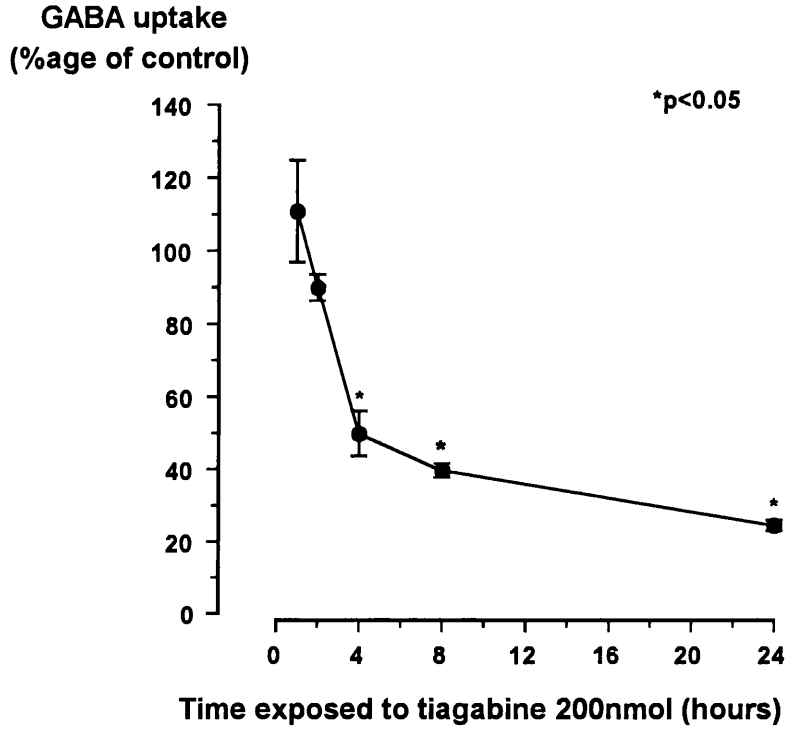


Figure 32: Mean GABA uptake (±SEM) in primary cultures of rat astrocytes.

Figure 33

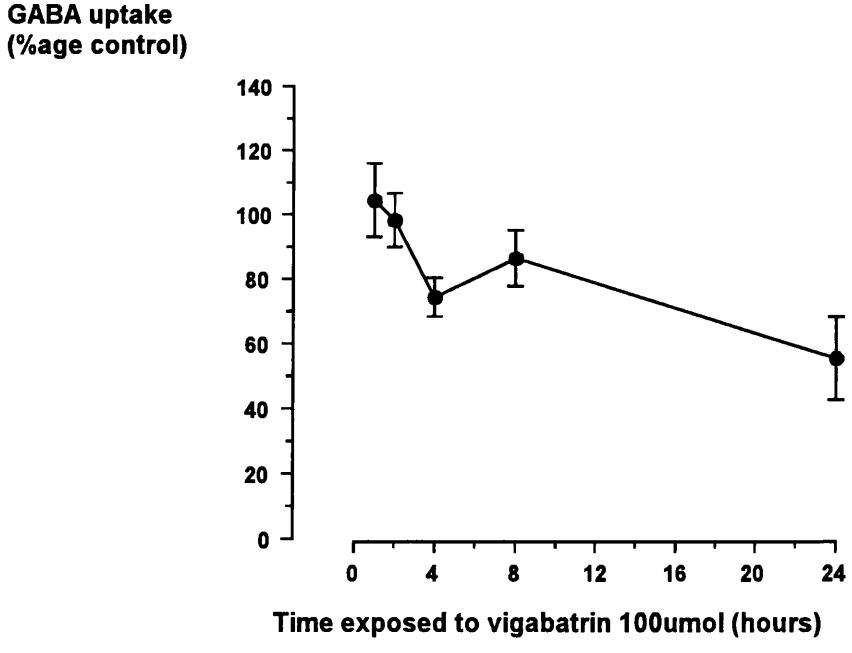


Figure 33: Mean GABA uptake (±SEM) in primary cultures of rat astrocytes.

Figure 34

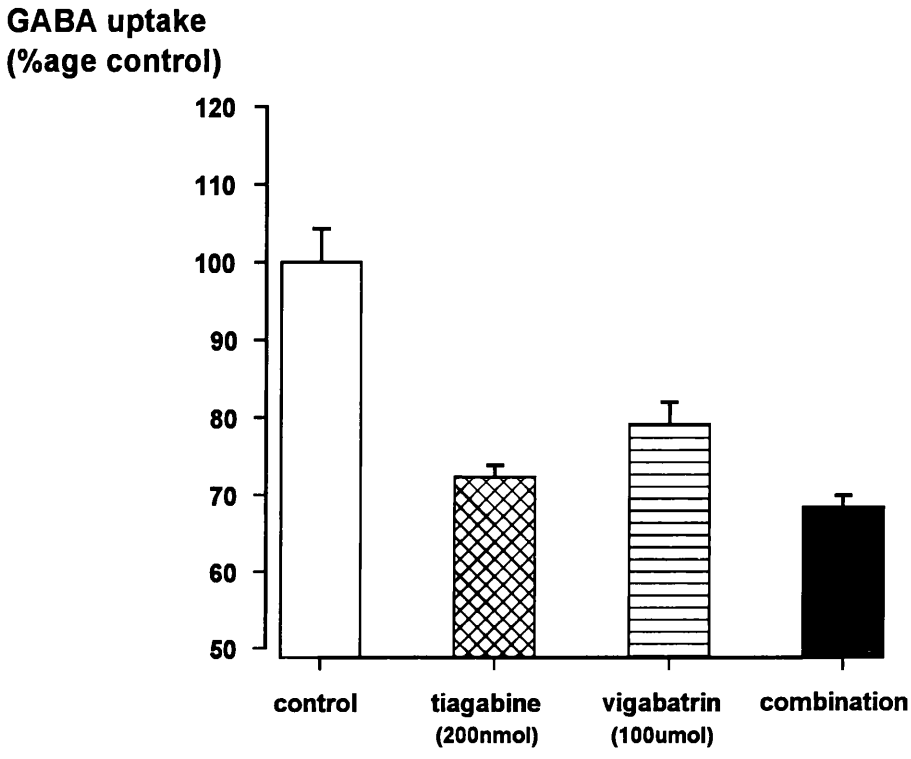


Figure 34: Mean GABA uptake (±SEM) in primary cultures of rat astrocytes exposed to GABAergic drugs at optimum concentrations for 4 hours.

Discussion

Both vigabatrin and tiagabine are significant advances in the treatment of epilepsy over the last 5 years (Leach and Brodie 1995). We have previously suggested that the combination of both drugs may be particularly beneficial (Leach and Brodie 1994), and this has also been noted by other clinicians (Dr Pam Crawford - Personal communication).

We have shown that the effect of tiagabine on GABA uptake is dose dependent. The concentration-effect profile would appear to be U-shaped, which makes determination of an IC50 impossible. A similar U-shaped curve has been reported using tiagabine in animal seizure models (Nielsen et al, 1991). Why higher doses of tiagabine are ineffective is unclear, although they may be cytotoxic to some degree. No sign of cell death, such as a decrease in protein yield was seen at any dose during these studies.

The delay in initiation of tiagabine-related GABA uptake inhibition was surprising. In whole animal studies, the anti-seizure effects are soon evident following parenteral administration (Nielsen et al, 1991). Further work is warranted soon after tiagabine administration to primary cell cultures to investigate this anomaly.

The demonstrated inhibitory effect of vigabatrin on GABA uptake confirms our earlier work (Sills et al 1993). Strangely, this effect was present at doses between 100 and 250 umol, with no effect visible at higher doses. The effective concentrations are close to the IC50 for GABA-T inhibition in similar models (Larsson et al, 1986). This secondary action might partly explain the wide range of animal models in which GABA-T inhibitors are effective (Schechter et al, 1979), and may also explain the difference in the time of

optimal GABA-T inhibition and that of maximal anticonvulsant in animal seizure models (Bernasconi et al, 1988).

Given the lack of response at higher doses, the inhibition of GABA uptake by vigabatrin is unlikely to be a result of a simple competitive block, despite their structural similarity. Previous studies have suggested that vigabatrin is not a substrate for any GABA transporter, but is believed to enter cells via some high affinity uptake system (Grant and Heel, 1991). With the appearance of work defining multiple GABA uptake carriers (Borden et al, 1994) however, it is possible to speculate that vigabatrin may have an action at one or more of these specific targets. GAT-3 may be a more likely candidate, since a secondary substrate of GABA-T, β-alanine (Benuck and Lajtha, 1975) selectively blocks this carrier (Clark and Amara, 1994). The structural similarities of GABA, β-alanine and vigabatrin may facilitate a common binding at active sites of GABA-T and the GAT-3 GABA transporter.

Tiagabine and vigabatrin did show some additive effect on GABA uptake inhibition when given in combination. The drugs may have a similar site of action, since the increased effect is infra-additive. This may support the possibility that the uptake inhibition may occur at GAT-3, since tiagabine has a minor role in blocking this transporter (Borden et al 1994).

Conclusions

Clinical experience with tiagabine had suggested that it's effect may be enhanced in patients already on vigabatrin therapy, and further studies are underway to investigate this. This effect of vigabatrin on GABA uptake may be

a partial explanation for the usefulness of this combination.

Further characterisation of vigabatrin's GABA uptake blocking properties may help to determine its relative importance as an additional mechanism of action. This is one situation where an improved definition of each drug's biochemical actions may pave the way towards a more rational and beneficial approach to polypharmacy.

One of the deficiencies in the study is the lack of investigation into the effect of these drugs on the levels of cortical glutamate. Further work directly correlating this would be justified, particularly following administration of vigabatrin. One other direction in which research should be undertaken is in the investigation of smaller doses of vigabatrin and tiagabine. Since whole brain biochemistry is changed significantly by combined low doses where the drugs used singly are ineffective, would the combined use of low dose have significant GABA-uptake blocking activity?

Clinical study of the efficacy and tolerability of adjunctive gabapentin in refractory partial seizures.

Cognition and gabapentin: a double-blind, placebo-controlled study in refractory epilepsy

The doses of gabapentin used in previous double-blinded studies are lower than would now be commonly used in clinical practice, and in these trials most adverse events reported with gabapentin usage are transient and minor (Browne 1993). The most common side effects include somnolence, dizziness, ataxia and fatigue (Browne 1993).

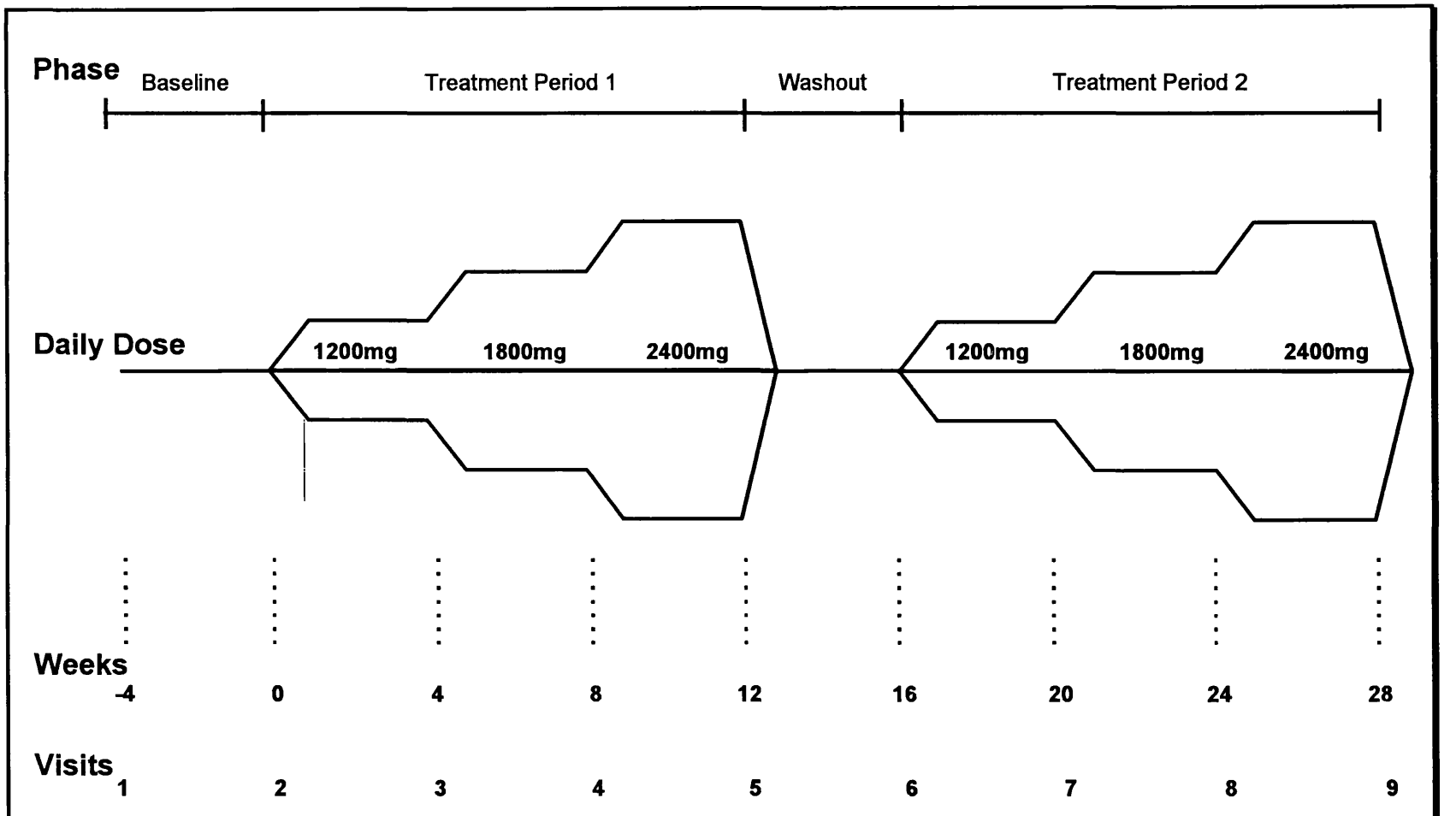
Using a battery of tests, we have previously shown that treatment with other anticonvulsants can alter psychomotor and cognitive testing in a dose-related manner (Macphee et al 1986, Brodie et al 1987, Gillham et al 1988, 1990, 1991, 1993). If newer AEDs such as gabapentin are to attain a satisfactory market share, they will have to prove that they are at least as well tolerated as the older agents.

Aims

The aim of the study was to assess the efficacy and tolerability of different doses of gabapentin in patients with treated refractory epilepsy.

Methods

Twenty seven patients were recruited from the Epilepsy Research Unit at the Western Infirmary in Glasgow (Table 13). Details of the study were approved by the local Ethical Committee and all patients gave full, informed, written consent to their participation. All had epilepsy refractory to at least one of the



At each visit, the following tests were done: 1) Cognitive function tests 2) Patient Self-assessment questionnaire 3) Plasma / serum concentrations of antiepileptic drugs

currently available AEDs. Seizures were localisation-related, and all patients had a history of adequate compliance, were able to follow dosage instructions, and were able to have their seizures fully documented for the duration of the trial. All patients were receiving at least one standard AED at a dose that had remained stable for at least three months.

Protocol

The study was a double-blind, random-order, crossover, comparison of three increasing doses of gabapentin with matched placebo (Figure 35). One month after a screening visit, patients were entered into the first 12-week treatment phase. Psychomotor and cognitive testing was carried out every four weeks, prior to the increase in gabapentin/placebo dose.

After three dose levels had been tested during the first treatment phase, patients entered a 4-week washout period prior to commencing the second treatment phase. Patients were given individual appointment times, and each visit was at a similar time of day to minimise variability in drug levels at each visit. Throughout the trial, tablet compliance was checked by tablet counts and on history from the patient at each visit. On completion or following premature withdrawal from the trial, each patient was given the opportunity to continue gabapentin treatment.

Test Battery

A total of eight tests and three self-assessment scales were administered at each four-weekly visit. The tests included three psychomotor tests, and five

memory tests, while the self-assessment scales involved SEALS questionnaires, an AED related symptom score, and a visual analogue score (VAS) for drowsiness.

Psychomotor tests

The decision time was the time in milliseconds to respond to a light coming on by removing the finger from the base button in a choice time reaction task. The mean of 30 trials was recorded. *The movement time* was the time in milliseconds to move the finger from the base button to extinguish a light. The mean of thirty trials was recorded. *Decision and movement times* were carried out using the Leeds psychomotor tester.

In the *threshold detection test*, an array of small rectangles was displayed on a computer screen. After a brief time, an extra rectangle was added to the array. The patient was required to indicate which it was. The 'threshold' was the minimum time gap in frame units between the presentation of the arrayand the additional stimulus that the patient required to perceive that an extra rectangle had been added. This test was administered using an Apple IIe microcomputer.

The stroop test assessed the patients decision making and flexibility. Patients were given a card with a list of words written on it, printed in inks of varying colours. They were then asked to go through the lists, stating the colour of ink used in each word, ignoring what the word says. The number of colours stated correctly within two minutes of starting was the basis of the score.

Memory tests

All memory tests except for the paired associated learning, were performed at every visit. *The forward digit span tes*t measured the maximum number of digits the patient could recall immediately following oral presentation. Patients were allowed two trials at each level. *The backward digit span* measured the maximum number of digits that the patient could recall in reverse order immediately following oral presentation. Both digit span tests are discontinued when two tests at the same level were failed. *The forward visual span* measures the maximum number of squares correctly reproduced in sequence as demonstrated by the examiner. *The backward visual span* assesses the maximum number of squares correctly reproduced in reverse order to that demonstrated by the examiner. The visual span tests are terminated when two consecutive tests are failed.

The paired associate learning test gives a score up to 18 depending on the number of attempts required by the patient to name three sets of correctly paired words. In the *Rivermead behavioural memory test* the 'screen score' of this standardised psychometric battery was used.

Self reporting scales

SEALS I involves 50 questions which the subject answers in order to assesses subjective feelings of specific symptoms such as cognitive slowing, dysphoria, irritability, fatigue, and worry. The period under assessment was the 7-day period immediately prior to the visit.

To calculate the *Drowsiness score* patients were asked to demonstrate, with

the help of a visual analogue scale, their degree of alertness. The measured distance along the line chosen by the patient was recorded at each visit.

The *AED-Related Symptom Score* is constructed from patients' responses to a four-point grading score for 10 adverse effects (dizziness, dry mouth, flushing, headache, nausea, double vision, ankle swelling, tremor, unsteadiness, and palpitations). Only six are associated with AED use, the other four being controls.

Drug Assays

Carbamazepine, sodium valproate, phenytoin and phenobarbitone concentrations were measured by enzyme immunoassay (Emit, Syva, Palo Alto, USA). Vigabatrin was extracted from plasma into ethylacetate, heated with dansyl chloride at high pH to form a fluorescent derivative, and measured by HPLC with phenylGABA as internal standard. The interassay coefficient of variation (CV) over the range 1-100mg/L was 5% and the lower limit of detection was 0.1mg/L. Lamotrigine was extracted into ethinylacetate from plasma with 2M sodium hydroxide and measured by HPLC with BWA 725C (Wellcome Laboratories, UK) as internal standard. The interassay CV over the range 0-5mg/L was 6% with a lower limit of detection of 0.25mg/L. A recently devised gabapentin assay using HPLC with fluorimetric detection (Forrest - in press) was utilised in our laboratories. The inter- and intra-assay variations of this method were 3.8% and 2.6% respectively at 5ug/ml, and the detection limits were 1 and 10ug/ml.

Table 13

Demographic data of evaluable patients in gabapentin study

Patient Number	Sex	Age	Duration of epilepsy (years)	Baseline seizure frequency (n/28 days)	Treatment	Seizure type
1	M	26	24	5	CBZ, VGB	CP / GTC
2	F	16	13	91	CBZ,	SP / GTC
3	F	37	36	42	CBZ, LTG	SP / GTC
4	M	35	10	0	CBZ, PHT	SP / GTC
5	F	27	19	5	VPA, PRM	SPCP
6	M	54	3	5	PHT	CP / GTC
7	F	31	17	8	CBZ, PHB	CP
8	M	38	24	7	CBZ, VGB	CP / GTC
9	F	25	23	212	CBZ, VPA	CP / GTC
11	F	38	36	6	CBZ, PRM	CP
12	M	35	18	0	VGB, LTG	CP / GTC
13	M	40	5	15	CBZ	SP / GTC
15	F	67	62	0	CBZ, CZP	CP / GTC
16	M	41	9	28	OCB,	SPCP/GTC
18	M	41	34	6	VGB, PHT	SPCP/GTC
19	M	46	7	3	CBZ	CP / GTC
20	F	25	13	71	CBZ, VGB	CP / GTC
21	M	44	2	7	VPA	CP / GTC
22	F	39	30	4	CBZ, VGB	SPCP/GTC
23	F	30	13	30	CBZ, VPA	SPCP
26	F	32	18	7	VPA	CP / GTC
28	F	39	34	8	CBZ, PHT	CP

CBZ = carbamazepine, VGB = vigabatrin, LTG = lamotrigine, PHT = phenytoin, PRM = primidone, PHB = phenobarbital,VPA = sodium valproate.
CP / GTC = complex partial with occasional secondary generalisation.
SP / GTC = simple partial with occasional secondary generalisation.
SPCP = Simple partial seizure evolving into complex partial.
CP = complex partial.
SPCPGTC = simple partial evolving into complex partial with occasional secondary generalisation.

Statistics

Statistical analysis was carried out by the biometrics unit at Parke Davis Ltd using the SAS statistical package. The primary measure of seizure control is the comparison of seizure frequency during active (gabapentin) treatment with that during placebo treatment phase. Seizure frequencies were normalised using a log transformation prior to analysis. Changes from baseline in individual cognitive function tests were analysed using the Wilcoxon signed ranks test. In addition to the analysis of individual tests, composite scores for memory and psychomotor performance were constructed by summation of the normalised scores for related assessments.

Composite memory and psychomotor scores were compared using analysis of variance. The five SEALS subscores and the visual analogue scale of drowsiness were explored using analysis of covariance with the baseline measure as covariate. Data for each dose level was assessed separately.

Results

Twenty of the 27 patients completed the study (Table 13). Five patients withdrew because of the onset of adverse events (4 on placebo, 1 on gabapentin), and two requested withdrawal due to lack of efficacy (1 each on placebo and gabapentin). Five of these patients withdrew prior to the onset of the second phase, leaving 22 patients who had been exposed to both treatment phases and were eligible for analysis.

Seizure frequency

The frequency of all seizures combined was significantly reduced by gabapentin treatment (p=0.02) throughout the active treatment period (Table 14). Of the 21 patients exposed to all doses, nine (43%) had their total seizure frequency reduced by at least 50% while on concomitant gabapentin. Efficacy was greatest at 1800 and 2400mg/day of gabapentin. Two patients were seizure-free throughout the active treatment phase with none responding similarly to placebo.

Seven patients experienced simple partial seizures without secondary generalisation during the study. Simple partial seizures were not significantly affected by gabapentin, although 2 patients (29%) had the seizure frequency reduced by at least half i.e. 'responded' to treatment. Complex partial seizures without secondary generalisation, noted in 17 patients, were also reduced on gabapentin, with 5 patients (29%) 'responding' to treatment, although the overall reduction did not reach statistical significance. Ten (59%) of 17 patients experiencing secondary generalised seizures 'responded' to gabapentin treatment, with the median monthly frequency of this type significantly reduced from 1 to 0.3 per month on gabapentin (p=0.01).

Table 14

Effect of gabapentin treatment on median frequency and interquartile range of each seizure type.

	n		Gabapentin	Placebo	P value
Simple Partial	7	Frequency Range	1.7 0-14.3	1.7 0-14.3	0.80
Complex Partial	17	Frequency Range	3.0 2.0-4.3	3.7 1.3-6	0.62
Secondary Generalised	17	Frequency Range	0.3 0.3-3.3	1.0 0.3-7.3	0.01
Total Seizures	21	Frequency Range	4.3 2.3-16.7	7.0 3.7-19.7	0.02

Neuropsychological assessment

No order effect was seen in any parameter of psychomotor or cognitive testing i.e. there was no improvement in the tests as the trial progressed.

Psychomotor testing

Composite scores of psychomotor function were not altered by gabapentin treatment (Table 15). Comparison of composite psychomotor scores and individual tests at each treatment level with the corresponding placebo phase fails to show any significant difference. Mean psychomotor function results during the placebo treatment phase were plotted against seizure frequency, showing an interesting negative correlation between frequency of ictal events and psychomotor capabilities (Figure 36) which reached statistical significance.

Table 15

Mean (SD) comparative psychomotor, memory and visual analogue drowsiness scores.

	Gabapentin	Placebo
Composite Psychomotor Score	0.0 (3.6)	0.0 (2.7)
Composite memory Score	0.2 (3.6)	-0.3 (3.6)
Visual Analogue Fatigue Score	43 (28)	39 (28)

Figure 36

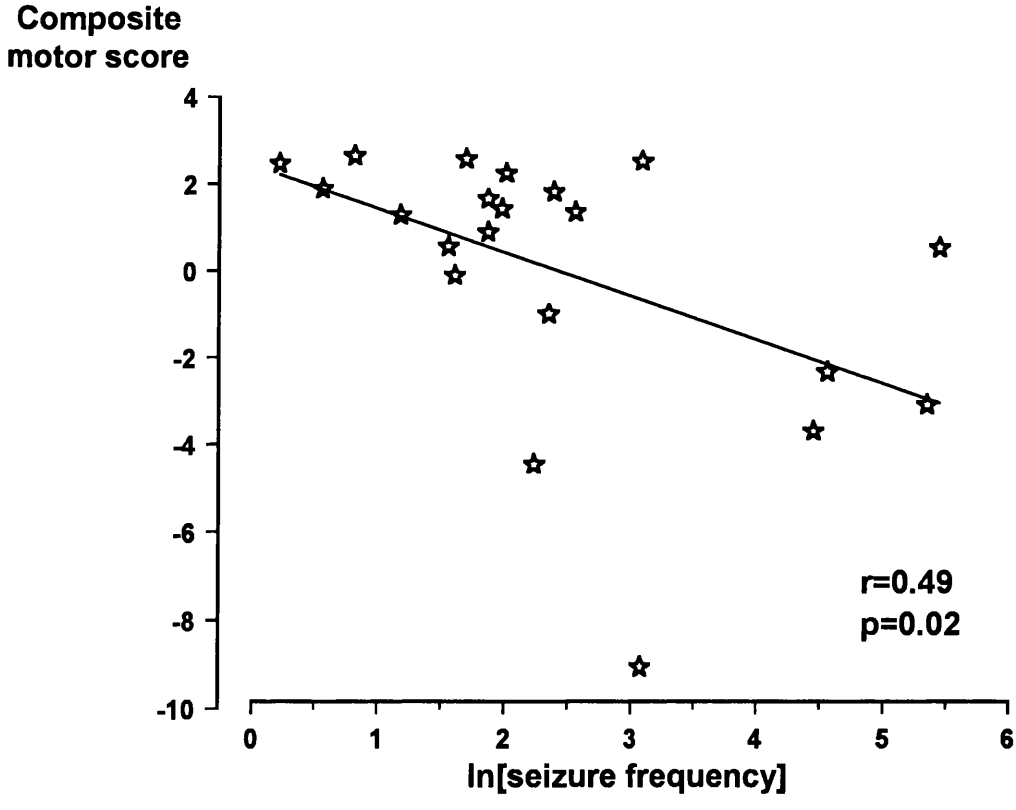


Figure 36: Correlation between seizure frequency and composite psychomotor scores throughout placebo treatment phase.

Memory testing

Composite memory scores showed no impairment on gabapentin (Table 15), even at highest dose. Comparison of individual test results between the two treatment phases showed only two tests to be significantly altered: the paired associate learning test being significantly improved on active treatment ($p=0.02$), while the backward visual was better on placebo treatment ($p<0.05$). No correlation was found between seizure frequency and memory function testing (Figure 37).

SEALS-1

SEALS scores were compared for each patient throughout both treatment phases. Further comparisons were carried out at each dose level and compared with the corresponding placebo dose level. Of the five subscores, none were significantly affected by gabapentin even at highest dose, although the increases in subscores for tiredness and cognition narrowly failed to reach statistical significance ($p=0.06$, and 0.08 respectively) (Table 16).

Fatigue algorithm

The VAS drowsiness score was significantly greater during treatment with 2400mg/day of gabapentin compared to the corresponding placebo dosing phase ($p=0.03$) (Table 15). Interestingly, there was a stronger, statistically significant correlation between the degree of fatigue reported and seizure frequency ($r=0.47$) (Figure 38).

Figure 37

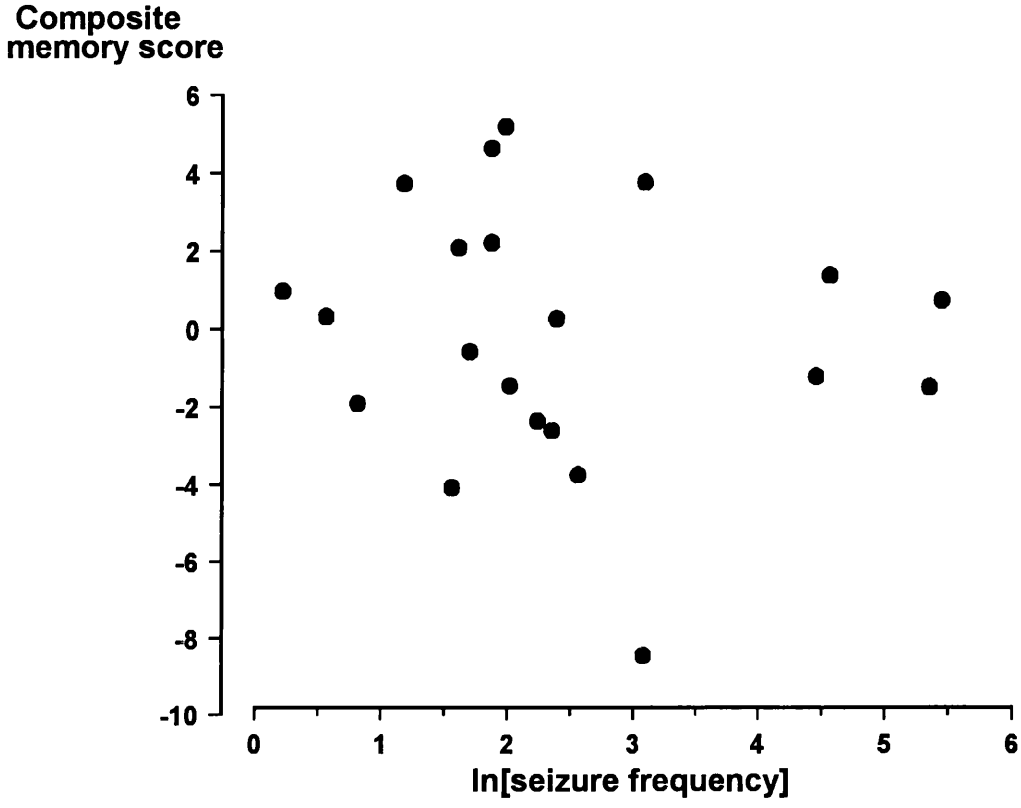


Figure 37: Correlation between composite memory scores and seizure frequency throughout placebo-treatment phase.

Table 16

Mean (SD) SEALS Subscores during treatment with 2400mg/day gabapentin or placebo.

	Gabapentin	Placebo	P value
Cognition	49 (12)	51 (13)	0.08
Dysphoria	17 (5)	17 (5)	0.25
Temper	12 (4)	12 (3)	0.61
Tiredness	13 (3)	14 (4)	0.06
Worry	10 (4)	10 (3)	0.58

Adverse events

Similar numbers of patients reported adverse events on gabapentin and placebo. Five patients withdrew because of the onset of adverse events, four while on placebo, and one while on gabapentin (because of a rash). Nineteen patients (79%) reported 47 adverse events on gabapentin, compared to the placebo phase where 15 patients (63%) reported 30 adverse events.

The AED symptom scores which were constructed showed a statistically significant change while on the highest dose of gabapentin ($p=0.006$). No significant differences were seen at the lower two doses. Interestingly, there was again a stronger correlation between AED symptom score (Figure 39) and seizure frequency ($r=-0.72$, $p=0.0002$) than with any form of drug treatment.

Drug Concentrations

The median plasma level of gabapentin increased with each dose increase: mean levels at 1200, 1800, and 2400mg/day were 4.7 ± 2.6, 6.8 ± 3.8, and 8.6 ± 3.3mg/L respectively. As would be expected from previous studies, there was no change in other AED levels following gabapentin treatment (Table 17).

Figure 38

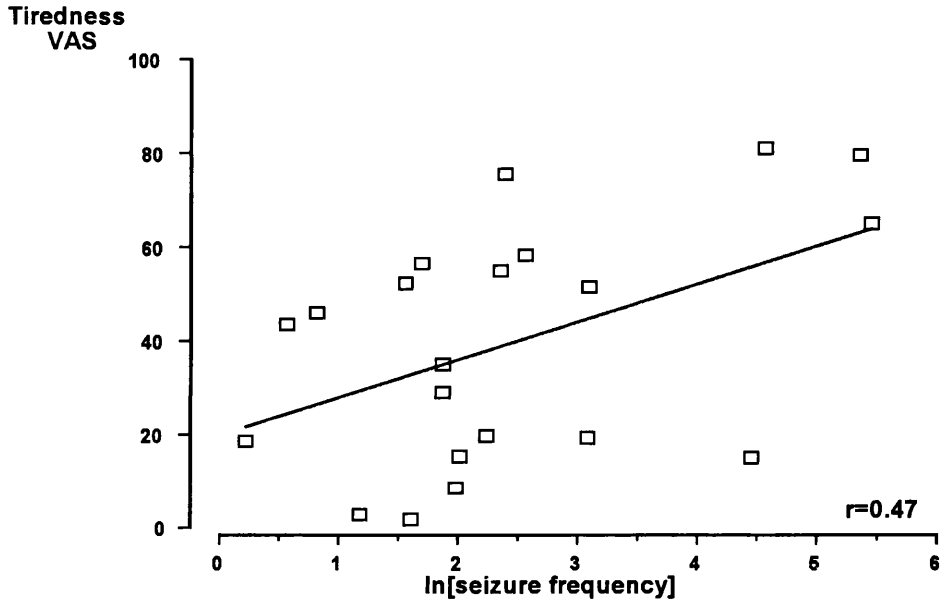


Figure 38: Correlation of VAS scores for fatigue and seizure frequency.

Figure 39

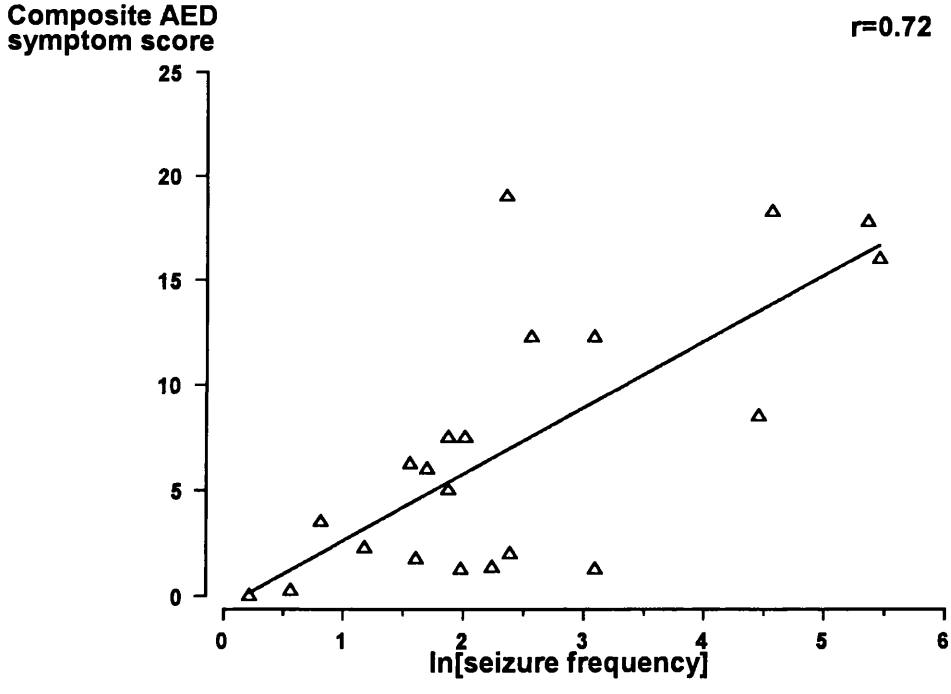


Figure 39: Correlation of composite adverse event score and seizure frequency.

Table 17

Mean antiepileptic drug concentrations (mg/L) during placebo or gabapentin dosing phase.

	n	Gabapentin	Placebo
Carbamazepine	16	8.7	8.0
Lamotrigine	2	6.6	6.1
Phenobarbital	1	27.4	26.1
Phenytoin	4	14.3	15.4
Primidone	2	9.6*	9.6
Sodium valproate	5	77.1	82.5
Vigabatrin	6	25.1	26.0

*Only one patient exposed to gabapentin and primidone.

Discussion

Cognitive function is an important issue in the treatment of epilepsy. Many patients complain of impaired memory and slowed thinking while receiving long-term anticonvulsant therapy, and the recent literature on psychomotor testing in epilepsy patients is testimony to doctors' attempts to quantify the extent of any drug-related cognitive impairment (Vermeulen and Aldenkamp 1995). This delineation of the higher mental effects of AEDs is vital: if the newer drugs are to thrive, they will have to prove to be better, or at least as well tolerated as the established AEDs.

This study has used higher doses of gabapentin than other double-blind trials. Despite the refractory nature of the study cohort, the response rates were comparable with those in the best of the efficacy studies (Anhut et al 1994, Crawford et al 1987, UK Gabapentin Study Group 1990, US Gabapentin study group 1992). As with other studies, the effect on secondary generalised seizures has been greater than on other seizure types (McLean 1995).

The lack of interaction with other AEDs confirms the findings of other studies (Hooper et al 1991, Radulovic et al 1994, Tyndel et al 1994), and is another advantage of gabapentin. This is particularly true in a setting where for the moment at least, use in the UK is only as add-on therapy.

At doses high enough to produce a significant reduction in seizure frequency, gabapentin has demonstrated a relative lack of psychomotor or memory impairment. Findings of a lack of psychomotor adverse events with gabapentin are consistent with previous reports of gabapentin's clinical tolerability (Browne 1993). There were two measures of fatigue that were

increased with gabapentin treatment; the fatigue visual analogue scale was significantly increased throughout the treatment phase while the SEALS subscore for tiredness narrowly failed to achieve statistical significance. Although multiple comparisons have a higher chance of providing some isolated statistically significant results, the fact that the scores were measuring closely allied symptoms may lead us to believe that the findings are clinically as well as statistically significant. Other similarly designed trials (Gillham et al 1993, Macphee et al 1986) have shown that tolerance can develop to fatigue and drowsiness. Were the highest dose levels to be observed for longer, the same tolerance may have been observed.

The correlation between seizure frequency and both VAS tiredness and composite psychomotor testing is interesting. That patients with more frequent seizures have more symptoms and signs of impairment may arise from several factors. Whether this change in response represents the workings of an innately more 'damaged' brain, or the impairment secondary to more frequent seizures will be difficult to assess. That the correlation between seizure frequency and these two variables was much stronger than the correlation with gabapentin levels or gabapentin dosage may imply, however, that the paramount consideration in improving cognitive function should be a reduction in seizure frequency.

General conclusions and discussion

General discussion

The last five years have seen welcome additions to our pharmacological armamentarium. Prior to the late eighties there were five anticonvulsant compounds in common use in the UK. By the end of this century it appears likely that there will be up to eleven new AEDs licensed. Meanwhile, progress in neuro-imaging and epilepsy surgery also continues apace. In all of history, there has never been a time when we could survey the unconquered lands of refractory epilepsy with such optimism.

There has, with justification, been a great deal of interest in the new anticonvulsants. Despite their introduction, however, most people would agree that a significant proportion of our patients remain unsatisfactorily controlled. Where the new AEDs have probably been of most benefit is in reducing the number of adverse events experienced. If their efficacy continues to equal that of the established agents, then, and only then will their future be assured. If even our best newest agents leave some seizure types untouched, then how are we to increase the number of patients in whom full control is achieved? Given the nature of the new AEDs, perhaps it is time to re-evaluate the role of combination therapy for epilepsy. As previously argued, the rational combinations will require a greater knowledge of the drugs' actions. Do the studies described in this thesis help us in this regard?

Vigabatrin

When tested in cultured cells and whole brain, vigabatrin demonstrated some effects other than GABA-T inhibition. GABA uptake was significantly reduced

in a dose-dependent manner. If vigabatrin increases the optimal GABA uptake inhibition in combination with tiagabine, then this may explain the possible additive effect noted when the two drugs are given together. When used in mice, the effective inhibition of whole brain GABA-T activity was demonstrated alongside an equally significant rise in GABA concentrations, and a comparable decrease in GAD activity.

Gabapentin

The double-blind clinical study has confirmed earlier impressions that at anticonvulsant doses, even as add-on therapy, gabapentin has little discernible effect on psychomotor function or on memory. Interestingly, under controlled conditions in our study, there was a significant relationship between seizure frequency and performance in psychomotor testing.

The effects of gabapentin on whole brain biochemistry failed to show any increase in GABA concentrations or GAD activity, but did show significant decreases in GABA-T activity and a trend towards a reduction in glutamate concentrations after multiple dosing. When placed alongside other in-vitro work suggesting positive effects on GABA release and GABA concentrations, it seems likely that gabapentin depends, at least in part, on its GABAergic effects for its anticonvulsant activity.

Tiagabine

The effect of tiagabine on GABA uptake in cultured astrocytes was quantified, and the dose-response curve found to be 'U'-shaped. Tiagabine had no effect

on cortical biochemistry when used alone, aside from an increase in GABA-T activity of dubious significance. When used in combination with vigabatrin at low dose, there was an increase in GABA concentrations demonstrated which was not obvious with either drug used alone. At medium and high dose, there was no significant difference between the biochemical effects of vigabatrin alone and in combination with tiagabine.

Remacemide

The potential for interactions with the older AEDs was investigated in patients. Remacemide did not significantly affect the concentrations of valproate, or the carbamazepine metabolite CBZ-E. While on oral remacemide, there was a trend towards an increase in phenytoin concentrations, which failed to reach significance except in the case of the trough concentrations during the multiple dose treatment phase. In a minority of patients on carbamazepine, co-administration of remacemide hydrochloride significantly increased concentrations of the baseline AED. These interactions may be important, but are unlikely to be a significant impediment to the use of remacemide alongside established agents.

Among valproate-treated patients, the half-life and AUC of remacemide hydrochloride and its main metabolite ARL12495XX were similar to that of untreated volunteers. In contrast, patients pretreated with an enzyme-inducing AED had a significantly lower exposure to remacemide hydrochloride and ARL12495XX as measured by AUC, and this patient group may require higher doses of remacemide to achieve equivalent plasma concentrations of the two

compounds.

On assessment of the effect of ARL12495XX on GABA metabolism in mouse whole brain, some surprising findings were made. In addition to the effects on sodium channels and NMDA receptors, ARL12495XX would also appear to have effects on GABA metabolism. Multiple dosing with ARL12495XX appeared to cause a significant decrease in GAD activity and increase in GABA-T activity, although the clinical relevance of these actions is unclear.

How can we formulate a rational plan for development of rational polypharmacy?

1. Chance observation

This is traditionally the method by which useful combinations have been developed in other branches of medicine. So far, in terms of AEDs, no specific drug combinations have been shown to be better than any others. The combinations involving the newer agents that have been (incompletely) tested are those that have been noted by chance to work in a limited number of patients (eg. lamotrigine and valproate, vigabatrin and tiagabine).

All new AEDs have to undergo extensive clinical testing as add-on therapy. It would be efficient, and potentially productive if pharmaceutical companies testing new AEDs as adjunctive therapy were compelled to carry out further analyses to assess the relative benefits in groups of patients receiving different baseline AEDs. This type of analysis will not be without its bias, each drug being used predominately in different seizure types, but it may provide some useful clues for future drug use. The pharmaceutical industry, would

argue that this would be impractical: every company is understandably keen that their new product is seen as a 'cutting edge' compound rather than a mere cofactor. Meta-analysis of large trials, or of a number of trials, however could have advantages with which even the pharmaceutical industry could sympathise. Such meta-analyses may, for example, help forewarn us at an earlier stage of any important pharmacokinetic interactions involving the new compounds.

If we are to rely on clinical observation to give us hints as to ideal drug combinations, then we need regular reviews to improve our yield. For this reason among others, it would be helpful if the data upon which drug licensing applications are based were made available at an early stage in peer-reviewed journals. On researching the chapter on the new AEDs, it became apparent that there was a paucity of freely available clinical and pre-clinical data, even for those drugs whose licence had been granted. It is far from ideal that clinical and biochemical data of compounds on general release have to be gleaned from journal abstracts pending full publication of the study data. It is unrealistic, and probably unhealthy, for our understanding of such drugs to be based solely on the subjective, and often selective data provided by the pharmaceutical industry.

2 *Rational planning of useful combinations*

In this scientific age, it would be sensible to base our choice of AED combinations on the information available to us. With our increasing knowledge of neurophysiology and neurobiochemistry, is it more likely that we

should be able to make that choice?

Perhaps not yet. Despite the fact that we are better informed than ever before, there are still large gaps in our knowledge. It would have been naive to assume, for example, that remacemide would have no effect on the GABA shunt merely because it had already proven to have effects on glutamatergic neurotransmission. What we learn from this experiment may be that in terms of basic mechanisms of action of the AEDs, nothing should be taken for granted.

How will we determine what is best for our patients?

The knowledge of basic mechanisms of action, however complete, does not answer all of our questions about anticonvulsant treatment. Even if *all* the neurochemical and neurophysiological effects of *all* AEDs can be elicited, will that help us to decide how drugs should be paired? Should compounds be combined with drugs on the same side of the fence (eg two GABAergic drugs) or should we be using each one alongside others with actions on different systems? Some would argue that multiplicity of action is what has bedevilled the use of the older AEDs: according to this view, we should be searching for a drug that has one single anticonvulsant effect. In this view, combining drugs with different actions merely simulates the administration of an established drug.

In answer, having a combination of drugs producing a variety of effects is an improvement on having a single multi-active drug. The combination of more specific drugs offers a greater flexibility of manoeuvre in response to any

clinical improvement or the onset of adverse events. For a patient on lamotrigine / valproate combination, for example, the relative doses would be determined by the patient's response. If weight gain is a problem, valproate dose could be adjusted, whereas the temporary reduction of lamotrigine dose may suffice in the prevention of headache.

Just as importantly, could we combine drugs according to their biochemical 'side effects'? The pairing of vigabatrin and gabapentin, for example may allow for a greater inhibition of GABA-T with little GAD inhibition, so perhaps removing the ceiling to effective dosing of vigabatrin's efficacy (McKee et al 1993), or minimising any adverse events (Grant and Heel 1991).

Knowledge of the AEDs' biochemical effects may also warn us of combinations to avoid. It would be interesting to assess the effect of a remacemide / vigabatrin combination on whole brain GABA-T. Would this combination have any efficacy in animal seizure models? Would the dual depression of GAD activity produce a high incidence of side effects?

While we concentrate on the novel drugs' actions in in-vitro experiments, it is important that we do not forget the established AEDs. That lamotrigine and valproate combine so effectively is probably *more* than a result of the well-recognised pharmacokinetic interaction . Are there any particular aspects of lamotrigine's action(s) that mesh particularly well with the actions of valproate? Further work on the established AEDs to further delineate their neurophysiological effects would be useful, not only in terms of helping to formulate treatment plans, but also in helping to augment our knowledge of

the basic processes underlying epileptogenesis.

Synergy is an attractive proposition for doctors in all areas of medicine. To have drugs producing effects in combination that are supra-additive seems to be the biological equivalent of getting something for nothing. Once a potentially beneficial combination comes to our attention, by whatever means, how can we test its efficacy and tolerability, or determine if any synergism is effected? Pharmacologically, use of the isobologram first espoused by Loewe in 1953, may be the most effective way forward (Mawer and Pleuvry 1995) (Figure 40). The straight line isobologram (**A'**) charts doses of each drug

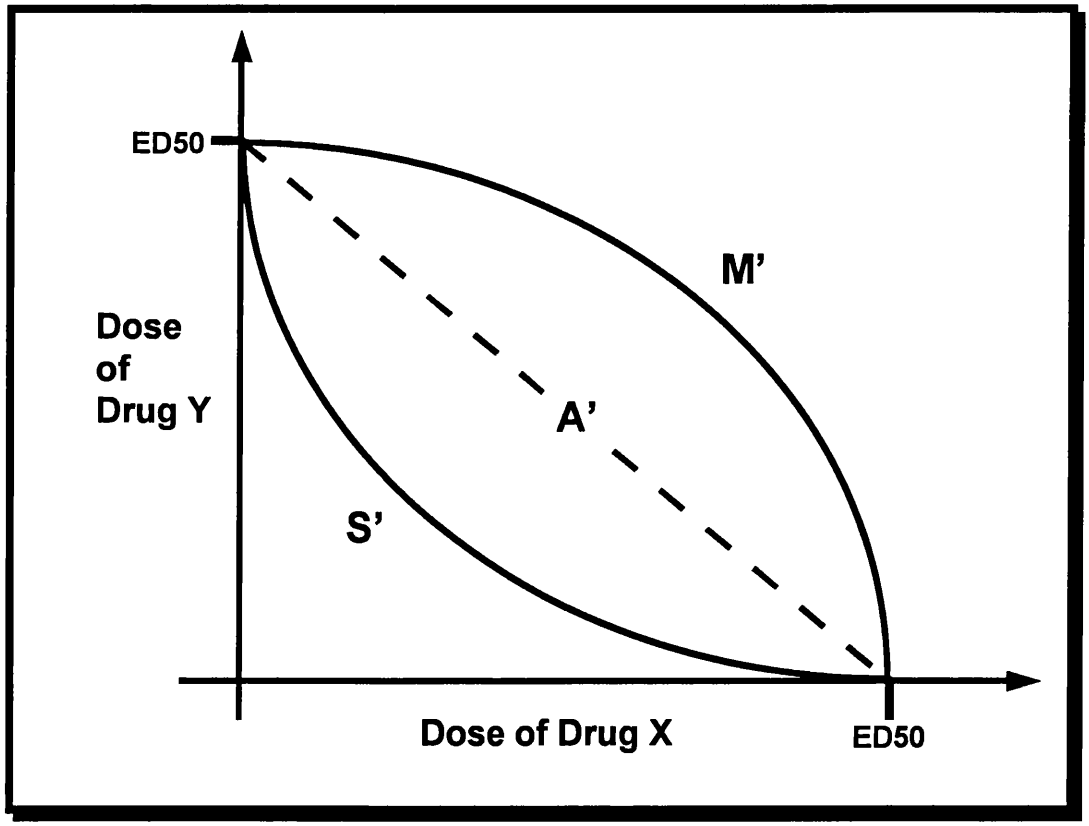


Fig 40- (Isobologram modified from Mawer and Pleuvry, 1995)

required to exert a particular effect if their actions are additive. If there is synergy or potentiation, the doses required to produce the same effect will be lower, and the curve for potentiation (**S'**) will be below the straight line. If the

drug effects are antagonistic, the doses of each drug required will be higher, and the curve (**M'**) will be above the line.

In theory this principle is useful, and in practice, it has been used (unsuccessfully) in the assessment of possible potentiation between felbamate and phenytoin (Swinyard et 1989). There are no clinical examples where isobolograms have been used to look at seizure effects, but in patients, isobolograms were used to examine the additive hypoglycaemic effect between salicylates and sulphonylureas. One of the problems in patients would be the determination of these 'equivalent' doses. Since epilepsy study populations are so variable, it would be difficult to decide which dose of valproate was equivalent, for example, to a specified dose of lamotrigine. Any pharmacokinetic interactions would have to be compensated for using blood levels, and yet there is no good correlation between serum levels of lamotrigine and its clinical efficacy.

In simpler terms, controlled clinical trials have been suggested (Richens 1995) which use a double-dummy design, comparing each drug singly with placebo, and with a combination of the two active treatments. Having four treatment arms, these trials will require large numbers of patients, one quarter of which will be receiving placebo. In most countries except the USA, there seems to (rightly) be some problems with placebo-controlled monotherapy studies. Elsewhere there may also be ethical problems if either of the drugs is not licensed for use as monotherapy. Where a drug dose differs between arms because of pharmacokinetic interactions (eg lamotrigine and valproate), this dose variance could cause some difficulty in interpreting the trial results.

There has been a fashion for recent reviews to assess the role of drug combinations in the treatment of epilepsy (Schmidt and Gram 1995, Richens 1995, Lammers 1995). That opinion in the last twenty years has tended to oppose combination therapy has also meant that little work went into planning of rational polypharmacy even before the introduction of the newer AEDs. While there are many reasons why the established AEDs don't mix well, it is sad, to say the least, that little effort has been made to provide an objective basis to support the use of various drug combinations.

Suggested Combinations for future investigation.

We now have a shortlist of combinations which would appear to have particular promise.

- Valproate \ lamotrigine

The clinical benefits of valproate\lamotrigine has been widely noted, and further investigation of this additive effect is essential. Both drugs are licensed for use as monotherapy in the UK, and there should be no ethical problem in having some patients on either of these drugs as monotherapy. The pharmacokinetic interaction between the two, however, may necessitate the provision of an unblinded observer to intervene where necessary, ensuring that plasma levels of lamotrigine, in particular, are comparable between groups.

- Lamotrigine and Vigabatrin

This is one of the few drug combinations tested under controlled clinical conditions that exclusively involves new AEDs. Previously, before lamotrigine had been granted approval for use as monotherapy, there were logistical problems in testing this combination. Stolarek et al (1994) used a placebo-controlled design and concluded that addition of a new drug was better than placebo. Further work by Schapel et al (1993), however confirmed the suggestion that the combination may have particular benefit. Studies of these two drugs in animal seizure models are being performed in our unit to test the efficacy against PTZ and MES-induced seizures in mice. Despite the efforts of the investigators mentioned, no properly controlled studies have been carried out which gives any scientific evidence of synergism.

- Vigabatrin and tiagabine

Our in vitro work, alongside clinical observations in our unit and further afield (Dr Pam Crawford - personal communication), would suggest that an investigation of the use of combined vigabatrin and tiagabine may yield some interesting results. Tiagabine will probably be granted a license for use in the UK in the near future, which would ease the logistical problems in organising this, although the lack of a monotherapy license for either drug would mean that the combination may initially at least be used only in addition to other AEDs.

- Gabapentin and vigabatrin

No clinical reports have yet been made to suggest particular clinical benefit in combination of these drugs, but our whole brain work may suggest that the combined use may lead to more complete inhibition of GABA-T activity while GAD inhibition may be kept to a minimum. Again, once one or either of these drugs gains a monotherapy license, the required double-dummy trial will be easier to design.

- Gabapentin and Tiagabine

Unsurprisingly, given that gabapentin received its license in the UK after the main studies of tiagabine efficacy had begun, there are no clinical reports of the particular efficacy of this compound. In-vitro studies, however (Honmou et al 1995), and the results of our small pilot study, would appear to suggest that gabapentin and tiagabine could be of particular benefit in some patients. The results of our whole brain biochemistry work might suggest that combinations of gabapentin and vigabatrin could be of interest.

So far, most of these suggested combinations would appear to concentrate largely on the GABAergic system. Perhaps as more drugs of uncertain mode of action come into daily use, there may be more opportunity for manipulation of the excitatory system, which so far is only served by remacemide, lamotrigine, and to some degree, topiramate. The formulation of a rational plan for the use of AEDs in combination will take many years, and this thesis is only a very small step in that right direction.

Conclusions

The history of epilepsy therapy is littered with the remnants of chance observations. To a degree they have served us well, and for a majority of newly diagnosed patients we have drugs which will control their seizures with no or negligible side effects. For a significant minority, however, things are not so rosy. The advent of the newer drugs may help a few patients gain control of their epilepsy, but as the continued existence of specialist epilepsy clinics will testify, the challenge of refractory epilepsy will remain with us for the foreseeable future. There is much work to be done in investigating the new AEDs, including that needed to investigate their use in combination. The trials required will not be easy. They will present difficult logistical problems, such as the use of placebo as monotherapy, large numbers of patients, or possibly the exclusion of patients who have received either of the constituent drugs. Not least among these problems will be funding difficulties. At the moment, it seems unlikely that any major pharmaceutical company will be willing to

subsidise such work, and the cost of setting up such a huge multicentre study may be prohibitive for grant funding bodies. Until the clinical studies can get underway, then research into AED combinations may need to either involve smaller numbers or concentrate on preparing the groundwork by assessing the effects of the all AEDs in a wide range of in-vivo and in-vitro studies.

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Published papers and abstracts

Published papers relating to this thesis

(encl) i) New antiepileptic drugs: an explosion of activity
Leach JP, Brodie MJ.
Seizure 1995;4:5-17

(encl) ii) Lamotrigine: Clinical use
Leach JP, Brodie MJ. In:New Anticonvulsants - Fourth edition
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(encl) iii) A case of overdose of tiagabine
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(encl) iv) Determination of gabapentin in plasma by high performance liquid chromatography.
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(encl) v) Effects of tiagabine and vigabatrin on GABA uptake into primary cultures of rat cortical astrocytes.
Leach JP, Sills GJ, Majiid A, Butler E, Carswell A, Thompson GG, Brodie MJ. *Seizure* - In press

(encl) vi) Pharmacokinetic interactions between remacemide and carbamazepine: two drugs with active metabolites.
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Letters

Synergism between the GABAergic anticonvulsants
Leach JP, Brodie MJ.
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Papers in preparation

i) Interactions between remacemide and phenytoin
Leach JP, Grivan J, Jamieson V, Jones T, Richens A, Brodie MJ.

ii) The neurochemical effects of tiagabine and vigabatrin on whole brain biochemistry.
Leach JP, Sills GJ, Forrest G, Thompson GG, Brodie MJ.

iii) The neurochemical effects of gabapentin on neurochemistry in mice.
Leach JP, Sills GJ, Forrest G, Thompson GG, Brodie MJ.

iv) Remacemide - the effects on GABA shunt enzymes and substrates in mouse whole brain.
Leach JP, Sills GJ, Forrest G, Thompson GG, Brodie MJ.

v) Gabapentin and cognition: a double blind, plaebo controlled crossover study.
Leach JP, Girvan J, Paul A, Maton S, Brodie MJ.

Published abstracts

1) Interactions between Remacemide and the standard antiepileptic drugs.
Leach JP, Blacklaw J, Stewart M, Jamieson V, Oxley R, Richens A, Brodie MJ.
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2) Mutual pharmacokinetic interactions between Remacemide and Carbamazepine and Phenytoin but not Sodium Valproate in epileptic patients.
Leach JP, Blacklaw J, Stewart M, Jones T, Jamieson V, Richens A, Brodie MJ.
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4) Pharmacokinetic interactions between Remacemide and Carbamazepine: two drug with active metabolites.
Leach JP, Blacklaw J, Stewart M, Jones T, Jamieson V, Richens A, Brodie MJ. Epilepsia 1995; 36 (Supp 3): 163

5) Neurochemical effects of combined vigabatrin and tiagabine in rodent brain.
Leach JP, Sills GJ, Forrest G, Thompson GG, Brodie MJ.
Epilepsia 1995; 36 (Supp 3): 40

6) Effects of vigabatrin on GABA uptake in cultured rodent astrocytes.
Leach JP, Sills GJ, Forrest G, Thompson GG, Brodie MJ.
Epilepsia 1995; 36 (Supp 4): 72

Presentations

To the International League Against Epilepsy, UK branch Annual Meeting
London, May 5th 1995
"The effects of combining GABAergic anticonvulsants."
JP Leach, G Sills, G Thompson, G Forrest, A Carswell, MJ Brodie.

To the American Epilepsy Society, Baltimore, Md, USA. December 2nd 1995.
Effects of vigabatrin on GABA uptake in cultured rodent astrocytes.
JP Leach, Sills GJ, Forrest G, Thompson GG, Brodie MJ.

Seizure 1995; 4: 5–17

New antiepileptic drugs—an explosion of activity

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The low therapeutic index of established antiepileptic drugs coupled with a better understanding of the pathophysiology of seizure production has led to the development of a range of new therapeutic agents for the treatment of epilepsy. In this review, the three drugs recently licensed in the UK (vigabatrin, lamotrigine and gabapentin) are profiled, together with several of the more promising up-and-coming compounds (oxcarbazepine, felbamate, tiagabine, stiripentol, remacemide and topiramate). Future avenues for clinical research in the pharmacological management of the epilepsies involve their rational use both singly and in combination.

Key words: antiepileptic drugs; epilepsy; interactions; neuropharmacology; side-efects.

INTRODUCTION

Epilepsy is a common condition with a point prevalence of just under 1% of a given population[1]. There are, by implication, around 500 000 people in the UK alone who have epileptic seizures, fewer than 70% of whom will have them fully controlled with the currently available antiepileptic drugs[2]. Many patients, particularly those with underlying anatomical lesions, respond poorly to monotherapy and are treated with combinations of anticonvulsants that often cause disabling side-effects with only an outside chance of significantly improved seizure control.

Dose-related side-effects and idiosyncratic reactions are all too common with existing anticonvulsants. The tendency to produce headache, nausea, dizziness, drowsiness, cognitive impairment and other central nervous system side-effects is almost universal[2]. Their potential for teratogenicity[3,4] and for deleterious drug interactions[5] make their long-term use problematical. The last few years have been exciting times for epileptologists. There has been a rush of interesting new antiepileptic drugs into clinical development.

THE BASIS OF SEIZURES

Histologically, epileptic neuronal tissue often shows only non-specific changes such as gliosis or dendritic degeneration[6,7]. The biochemical interplay at the site of epileptogenesis is more interesting, and an understanding of the pathogenesis may lead us to alter successfully the subtle biochemical imbalances responsible for seizure generation and propagation. Recent advances in drug development have focused on the therapeutic potential of manipulating brain neurotransmitters.

Endogenous substances such as gamma aminobutyric acid (GABA), adenosine and glycine are thought to be of importance in inhibiting seizure spread[7]. Stimulation of the $GABA_A$ receptor results in an influx of chloride ions, stabilizing the neuronal membrane and preventing seizure activity. Work in primates has demonstrated a selective loss of such terminals in epileptogenic foci[8], implying a local deficit in inhibition. There is decreased binding, also, to the GABA/benzodiazepine receptor complex in temporal lobe lesions[9].

The role of the excitatory amino acids, especially glutamate and aspartate, has been well recognized for a number of years. Receptors for these and other dicarboxylic acids are present in altered density in patients with

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Table 1: Pharmacological profile of a range of new antiepileptic drugs

Drug	Mode of action	Half life (hours)	Elimination	Concentrations affected by...	Effect on other AEDs
Felbamate	? Glycine antagonist at NMDA receptor	14–22	Hepatic metabolism, some renal excretion	Enzyme inducers	PHT, VPA ↑ CBZ ↓
Gabapentin	? Inhibition of L-amino acid transport	5–7	Renal excretion	Nil	Nil
Lamotrigine	Blocks Na channels decreasing EAA release	25–29	Hepatic glucuronidation	Enzyme inducers and inhibitors	CBZ (pharmacodynamic interaction)
Remacemide	Non-competitive NMDA antagonist	3–5 (metabolite 12–18)	Hepatic metabolism (active metabolite)	Enzyme inducers and inhibitors	PHT, CBZ, ↑
Stiripentol	? Inhibition of GABA uptake and metabolism	Variable (saturation kinetics)	Hepatic metabolism	Enzyme inducers and inhibitors	PHT, CBZ, PB ↑
Tiagabine	Decreases GABA reuptake	4–13	Hepatic metabolism	? Nil	? Nil
Topiramate	Unknown	18–23	Renal excretion, some hepatic metabolism	Unknown	Unknown
Vigabatrin	Decreases GABA metabolism	5–7	Renal excretion	Nil	PHT ↓

NMDA, *n*-methyl-D-aspartate; EAA, excitatory amino acid; AED, antiepileptic drugs; GABA, gamma aminobutyric acid; PHT, phenytoin; VPA, sodium valproate; CBZ, carbamazepine; PB, phenobarbitone.

either generalized[10] or temporal lobe[11] seizures. This implicates the excitatory system in producing a micro-environment conducive to epileptogenesis. These processes and their interplay are important factors in determining the extent of the neurological instability underlying the ictal diathesis[12].

ESTABLISHED ANTICONVULSANTS

The last two first-line anticonvulsants to be introduced in the UK were carbamazepine in 1967 and sodium valproate in 1974. These are still the best drugs for controlling most types of seizures. There remains, however, a great deal of scope for improving our treatment of epilepsy. Fewer than 70% of patients are adequately controlled with antiepileptic monotherapy[13]. The addition of a second or third drug will provide substantial improvement in only around 10% of the remainder[2]. The more antiepileptic drugs the patient takes, the greater the incidence of adverse effects, particularly cognitive impairment and teratogenesis.

The modes of action of established antiepileptic drugs are multiple, complex and overlapping[14]. Their effects on the central nervous system are widespread and rather indiscriminate—a neuropharmacological blunderbuss rather than a laser! The introduction of three new anticonvulsants with novel mechanisms of action in the UK over the last 5 years has revolutionized our approach to refractory epilepsy. Vigabatrin, lamotrigine and gabapentin have led the way for a plethora of newer agents that are being extensively tested in scienifically-based studies world-wide.

This review will deal with the compounds that are breaking through in the routine management of epilepsy, and will focus also on the most promising drugs currently undergoing clinical trials. The pharmacological profiles of the anticonvulsants featured in this paper are summarized in Table 1. Their potential ranges of efficacy are outlined in Table 2.

NEW ANTIEPILEPTIC DRUGS

Vigabatrin

Vigabatrin was licensed in the UK for use as add-on therapy for refractory epilepsy in 1989. It is being increasingly used in Europe, Africa and Asia, but still awaits regulatory approval in North America.

Mode of action

Vigabatrin is a GABA analogue which acts as a 'suicide' inhibitor of GABA-transaminase (Fig. 1). It binds irrevocably to its target enzyme, and so facilitates GABA-ergic inhi-

and, thus, stabilizing neuronal membranes[28]. This, however, is unlikely to be the whole story[29]

Pharmacokinetics and interactions

Oral administration of lamotrigine leads to its rapid and near complete absorption. The elimination half-life is around 29 hours with metabolism largely by hepatic glucuronidation[30]. Interestingly, patients with Gilbert's disease have a longer half-life due to a decrease in the activity of the enzyme bilirubin uridine diphosphate glucuronyl transferase[31].

Lamotrigine does not itself induce or inhibit hepatic enzymes. Consequently, it has no influence on the metabolism of other lipid soluble drugs, including the oral contraceptive pill and warfarin. Sodium valproate has been shown to lengthen its half-life to around 59 hours, and the enzyme-inducing anticonvulsants, carbamazepine, phenytoin and phenobarbitone reduce it to around 12 hours[32]. There have been reports of symptoms of neurotoxicity (headache, nausea, dizziness, diplopia, ataxia) in patients taking carbamazepine in whom lamotrigine has been introduced[33]. These disappear when the dose of either drug is reduced. This is thought to be the clinical representation of a pharmacodynamic interaction[34].

Efficacy and tolerability

Ten double-blind, placebo-controlled, add-on studies have been carried out with lamotrigine[35–44], nine of which reported success against partial seizures with or without secondary generalization. Many open studies have confirmed these findings[45]. Clinical experience also suggests that lamotrigine is effective for the primary generalized epilepsies[46]. There is anecdotal evidence too to support its value in Lennox–Gastaut syndrome[47]. These observations have not yet been substantiated in controlled clinical trials.

Lamotrigine is a well-tolerated drug, with skin rash being the most common reason for withdrawal. This occurs in approximately 3% of patients and depends on the rate of introduction of the drug[48]. Side-effects such as dizziness, headache, nausea and vomiting, ataxia, diplopia and tremor are other minor problems associated with lamotrigine administration.

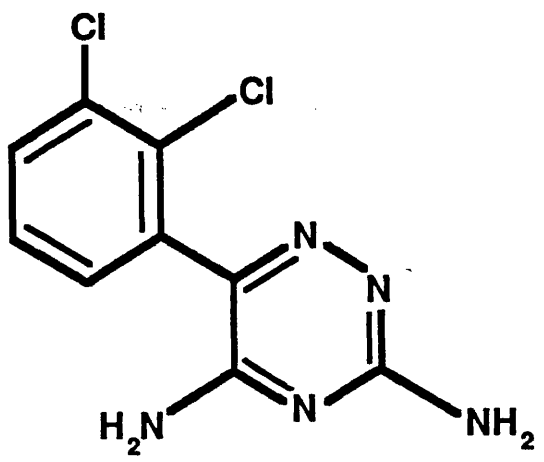

Fig. 2: Structure of lamotrigine.

Sedation is not a prominent manifestation of lamotrigine toxicity[49].

Double-blind trials to assess the usefulness of lamotrigine as monotherapy, using carbamazepine and phenytoin as comparators have just been completed. Preliminary results suggest equal efficacy to carbamazepine and phenytoin with better tolerability. Other comparative studies in children, in the primary generalized epilepsies and in the elderly are underway. The starting dose and titration rate will depend on existing treatment[50] when the drug is used as adjunctive therapy (Table 3). Lamotrigine is usually prescribed twice daily, but a single daily dose can be used if the drug is combined with sodium valproate or as monotherapy. A low, slow introduction schedule will reduce the likelihood of rash.

Table 3: Lamotrigine dosage and titration schedules

1. Add-on in treated adults and adolescents

	Valproate	*Others*
Weeks 1–2	25 mg alt die	50 mg daily
Weeks 3–4	25 mg daily	50 mg twice daily
Maintenance	50–100 mg twice daily	100–200 mg twice daily

2. Add-on in treated children

	Valproate	*Others*
Weeks 1–2	0.2 mg/kg	2 mg/kg
Weeks 3–4	0.5 mg/kg	5 mg/kg
Maintenance	1–5 mg/kg	5–15 mg/kg

3. Monotherapy in newly diagnosed epilepsy

	Adults	*Children*
Weeks 1–2	25 mg daily	0.5 mg/kg
Weeks 3–4	25 mg twice daily	1 mg/kg
Maintenance	50–100 mg twice daily	2–8 mg/kg

Higher doses can be tried if seizures persist and the patient is tolerating the drug without complaint.

Gabapentin

Gabapentin, a chemical analogue of GABA, was intended to act as a GABA agonist (Fig. 3). It was thought too that, being hydrophilic, blood–brain barrier penetration would be facilitated.

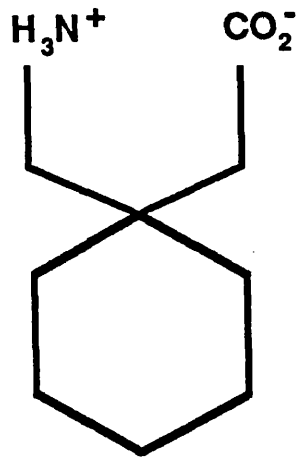

Fig. 3: Structure of gabapentin.

Mode of action

Interestingly, the anticonvulsant properties of gabapentin are not dependent on any direct action on the GABA-ergic system[51]. The drug appears to bind to a membrane site near the glutamate receptor, which may represent a transport system for L-amino acids[52,53]. A recent study suggests that gabapentin may also limit the rate of firing of sodium-dependent action potentials[54].

Pharmacokinetics and interactions

Oral dosing of gabapentin results in rapid absorption, and the drug has a bioavailability of 60%. A saturable transport mechanism in the gut explains the lack of proportionality between increased doses and levels in plasma[55]. Maximum concentrations occur 2–3 hours after administration and the elimination half-life approximates 5–7 hours[56]. There is no significant protein binding, and the drug is excreted unchanged in the urine with clearance rates equivalent to those for creatinine. The lack of important drug interactions with gabapentin has been widely reported[57].

Efficacy and tolerability

A small, dose-ranging, double-blind cross-over study was the first to provide evidence for gabapentin's efficacy against partial seizures[58]. A clear-cut dose–response relationship in reducing partial and secondary generalized seizures has been demonstrated with the drug[59]. In a large double-blind, parallel group, placebo-controlled study involving 127 patients with drug-resistant partial seizures, 25% of these taking gabapentin experienced a reduction in seizure frequency exceeding 50%[60]. The therapeutic effect of gabapentin has been shown to persist for up to 24 months[61].

Adverse events with gabapentin are generally mild and transient[62]. The most common are somnolence, fatigue, dizziness and weight gain. Other problems include diplopia, headache, ataxia and nausea. No idiosyncratic reactions have been reported to date. Early reports are reassuring also regarding its lack of teratogenic potential[63].

The recommended schedule for prescribing gabapentin involves thrice daily administration. However, some patients appear to respond to a morning and evening dose. The drug should be introduced over the first week at low dosage (e.g. 300 mg twice daily) and thereafter can be increased more rapidly as necessary to a maximum of 2700 mg daily or thereabouts. Its use is not currently recommended in children under 12 years of age.

Oxcarbazepine

Oxcarbazepine, the 10-keto analogue of carbamazepine (Fig. 4), has been licensed for use in a number of countries worldwide. Its metabolism differs from that of the parent compound and this holds out the possibility of a more benign side-effect profile with fewer drug interactions[64].

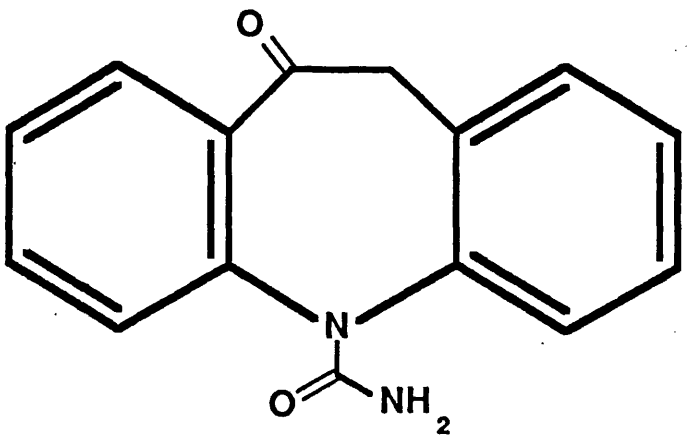

Fig. 4: Structure of oxcarbazepine.

Mode of action

The mode of action of oxcarbazepine is similar but not identical to that of carbamazepine[65]. Its major effect, therefore, is in preventing burst firing of neurones by blocking voltage-dependent sodium channels.

Pharmacokinetics and interactions

Oxcarbazepine is a pro-drug, the main active principle being 10,11-dihydro-10-hydroxy-carbamazepine which is largely eliminated by hepatic glucuronidation[63]. This moiety is 55% bound to plasma proteins and has an elimination half-life approximating 9 hours. Unlike carbamazepine, oxcarbazepine does not undergo autoinduction of metabolism[66]. The drug has no effect on the metabolism of other anticonvulsants[67]. However, the area under the concentration-time curve of the active metabolite is lower in patients taking carbamazepine, phenytoin and phenobarbitone than in controls suggesting a small, probably clinically irrelevant, induction effect of these drugs on its elimination[67,68].

Oxcarbazepine has less potential than carbamazepine to induce liver enzymes and so influence the metabolism of other drugs. It appears to induce selectively a single isoform of cytochrome P450, namely IIIA[69]. Volunteer studies have suggested that it does not interfere with warfarin metabolism[70], nor will cimetidine[71] or dextropoxyphene[72] inhibit its breakdown. However, decreased bioavailability of the oral contraceptive pill has been noted in some patients treated with oxcarbazepine[73].

Efficacy and tolerability

Several double-blind, add-on studies have confirmed comparable efficacy between oxcarbazepine and carbamazepine[74–76]. In addition, no difference in anticonvulsant effect was found between oxcarbazepine and carbamazepine in patients with newly diagnosed epilepsy[77]. Like carbamazepine, oxcarbazepine has no place in the treatment of absence seizures and myoclonic jerks[69].

Comparisons with carbamazepine have suggested a lower incidence of minor side-effects[75–77]. Those most commonly associated with oxcarbazepine are diplopia, nausea, headache, dizziness, drowsiness and ataxia[69]. Oxcarbazepine, like carbamazepine, has a propensity to cause hyponatraemia in some patients[78]. However, it produces fewer rashes than carbamazepine[79] and perhaps other idiosyncratic reactions[69].

Felbamate

Felbamate is a dicarbamate derivative (Fig. 5) which is licensed in the United States and has recently gained approval for use in partial seizures and Lennox–Gastaut syndrome in some European countries. It has been reported in animal models as having an unique profile of anticonvulsant activity, with little tendency to cause neurotoxicity[80].

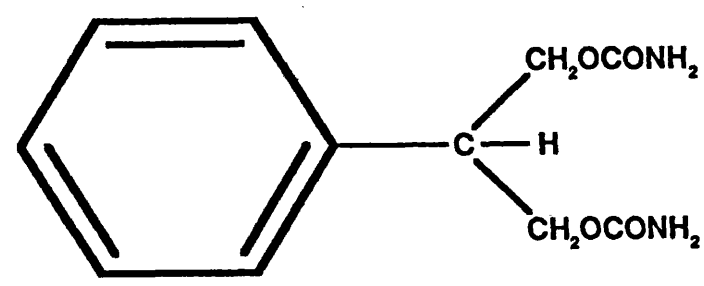

Fig. 5: Structure of felbamate.

Mode of action

Felbamate has an interesting effect in decreasing the binding of ligands at the glycine site of the *n*-methyl-*d*-aspartate (NMDA) receptor[81]. This implies that it may act as a glycine antagonist, possibly diminishing the deleterious influence on neuronal function of excitatory amino acids. Several other modes of action have also been suggested[82].

Pharmacokinetics and interactions

Oral administration of felbamate leads to complete and rapid absorption. Following a single dose, the half-life ranges between 14 and 22 hours in epileptic patients[82]. A proportion of absorbed drug is excreted unchanged in the urine, with the rest undergoing hydroxylation and conjugation in the liver.

On addition of felbamate, phenytoin, valproate and phenobarbitone levels will rise by around 20–30%, whereas serum carbamazepine will fall by a similar amount[83]. However, this latter effect is offset by greater production of the active metabolite, carbamazepine 10,11

epoxide[84]. Enzyme-inducing antiepileptic drugs will increase the rate of clearance of felbamate[85]. The effect of valproate on felbamate levels is less clear-cut[86].

Efficacy and tolerability

The efficacy of felbamate has been established in six major clinical trials involving around 370 patients[87–92]. Five of these were undertaken in patients with partial seizures with or without secondary generalization. The final piece of evidence consists of a major placebo-controlled trial in 73 children with Lennox–Gastaut syndrome, each of whom had at least 90 atonic or atypical absence seizures per month despite treatment with one or two established antiepileptic drugs. Felbamate reduced the frequency of atonic seizures as well as atypical absences[90]. Preliminary results in the primary generalized epilepsies are also promising. Long-term efficacy data with the drug, however, are still limited, although early observations are encouraging[93].

The most frequent side effects with felbamate originate from the central nervous system and include diplopia, dizziness, headache, nausea and vomiting, diarrhoea and ataxia[82]. Weight loss of around 5% body weight has been reported in some patients, probably secondary to anorexia and nausea. Both insomnia and somnolence have been associated with felbamate administration[83].

Tiagabine

Tiagabine, a nipecotic acid derivative (Fig. 6), has a powerful anticonvulsant effect in animal seizure models[94].

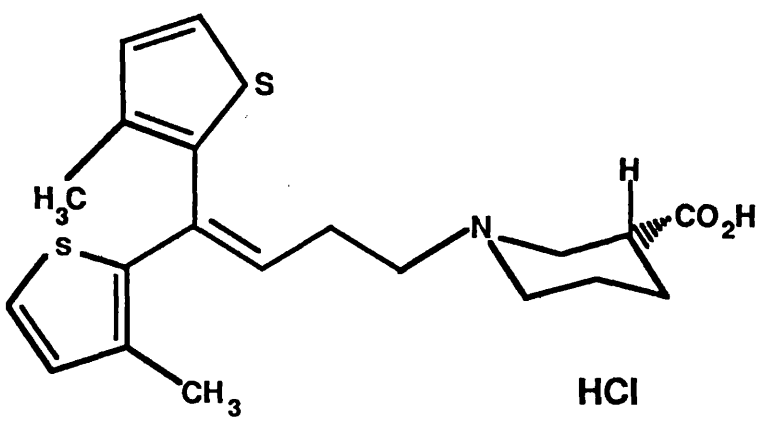

Fig. 6: Structure of tiagabine.

Mode of action

Tiagabine acts by inhibiting GABA re-uptake by glial cells and presynaptic neurones[95]. The resultant increase in synaptic GABA concentrations is thought to be responsible for its anticonvulsant action[96].

Pharmacokinetics and interactions

Tiagabine is well-absorbed orally with peak levels occurring around an hour after dosing[94]. The elimination half-life varies between 4 and 13 hours. Elimination is mainly by hepatic metabolism, which appears not to be influenced by concomitant anticonvulsant treatment.

Efficacy and tolerability

Double-blind studies suggest useful efficacy for tiagabine against partial and secondary generalised seizures[97,98]. Monotherapy trials are also currently being undertaken[99]. Side-effects so far have been largely confined to headache, dizziness and sedation.

Remacemide

Remacemide was designed to affect the excitatory component of epileptogenesis. It has recently entered phase III clinical trials in epileptic patients.

Mode of action

Remacemide is chemically unrelated to any other anticonvulsant (Fig. 7) and has been shown to be a weak, non-competitive NMDA antagonist[100]. At least part of remacemide's anticonvulsant action is thought to be due to its main metabolite, the desglycinate.

Pharmacokinetics and interactions

Oral dosing of healthy volunteers shows absorption to be rapid, peak levels being attained around 1 hour after administration. The parent drug has an elimination half-life approximating 4 hours, whereas the active desglycinate metabolite has a longer half-life

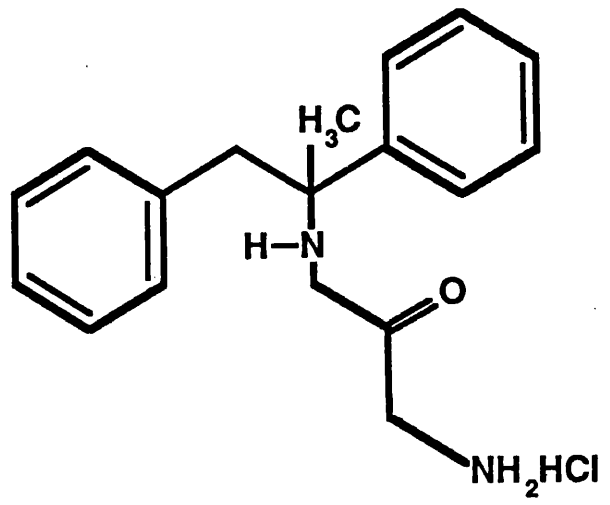

Fig. 7: Structure of remacemide.

in the region of 12–18 hours[100]. This difference in pharmacokinetics between parent drug and active metabolite is important in determining the optimum frequency of dosing.

Though not yet complete, interactions studies with remacemide suggest that it may act as an enzyme inhibitor, slowing the metabolism of both phenytoin and carbamazepine[101]. Clearance of remacemide is increased in patients taking phenytoin and carbamazepine compared with those treated with sodium valproate[102].

Efficacy and tolerability

Preliminary results with remacemide suggest useful efficacy against partial and secondary generalized seizures[103,104]. In common with other centrally acting drugs, lightheadedness, diplopia and dizziness have been reported as well as gastro-intestinal symptoms such as dyspepsia, nausea and vomiting. Aggressive behaviour has been noted on rare occasions. No evidence of idiosyncratic reaction to the drug has so far emerged.

Stiripentol

Stiripentol (Fig. 8) has been shown to be an effective anticonvulsant in many animal seizure models[105–107].

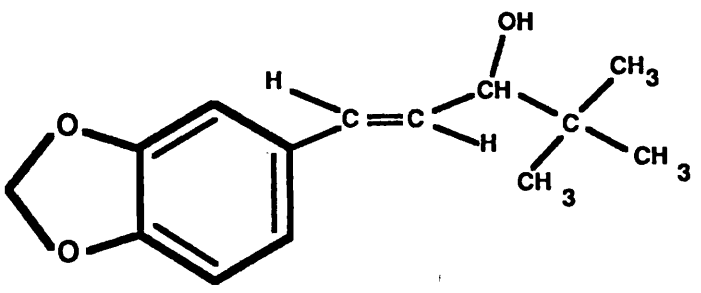

Fig. 8: Structure of stiripentol.

Mode of action

No precise mechanism of action has been determined, but laboratory studies suggest that stiripentol increases GABA levels either by inhibiting synaptosomal uptake or by decreasing its metabolism[108]

Pharmacokinetics and interactions

The drug is slowly distributed rendering its elimination curve multiphasic. It is highly protein bound and undergoes non-linear kinetics[109]. Stiripentol is biotransformed mainly by hepatic metabolism[110], and this can be accelerated by other antiepileptic drugs[111]. It strongly inhibits the metabolism of other anticonvulsants, causing a rise in concomitant phenytoin, carbamazepine, sodium valproate and phenobarbitone[112,113].

Efficacy and tolerability

Most of the studies carried out so far have been open label design in patients with partial seizures with or without secondary generalization[111,114]. A preliminary report supports efficacy in children with atypical absences[115]. Overall, stiripentol is well tolerated with only ataxia, nausea, vomiting and lethargy currently complicating its clinical use.

Topiramate

Topiramate (Fig. 9) is yet another antiepileptic compound, which is structurally distinct from all other comparable drugs!

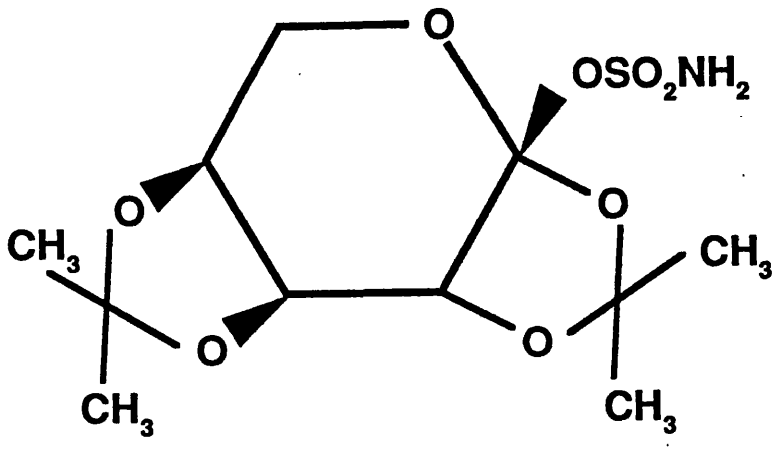

Fig. 9: Structure of topiramate.

Mode of action

As with many of the new anticonvulsants, this is presently uncertain. One study suggests that it may enhance GABA-mediated chloride ion influx[116].

Pharmacokinetics and interactions

Topiramate is slowly absorbed after oral dosing, peak concentrations in plasma occurring between 1 and 4 hours after administration. The drug is excreted largely unchanged in the urine, with an elimination half-life ranging from 19 to 23 hours[117]. Around 5% of a dose undergoes hepatic metabolism. Interactions with other anticonvulsants have still to be fully delineated.

Efficacy and tolerability

A number of double-blind, placebo-controlled studies have been completed with topiramate in adults with refractory partial seizures with or without secondary generalization[118,119]. These have shown a good response (defined as a reduction of >50% in seizure frequency) in more than half of the patients taking more than 200 mg of the drug per day[120,121]. Preliminary data also suggest efficacy in the Lennox–Gastaut syndrome[122]. Topiramate appears likely to be a valuable add-on treatment in refractory epilepsy[123].

The most frequently reported side-effects with topiramate include ataxia, cognitive dysfunction, dizziness, headache, nausea, nystagmus, tremor and visual disturbance. Less common problems include mood lability and weight loss. Animal toxicity studies suggest that topiramate may be teratogenic.

CONCLUSIONS

The recent developments in the pharmacotherapy of epilepsy are providing formidable challenges to the clinician. Which patients should receive which drug and for which seizure type? So far, most is known about lamotrigine and vigabatrin, as these have been in general use for some years. Felbamate and gabapentin have been licensed more recently in the USA and approved for licensing in some European countries. Oxcarbazepine, also generally available in some countries, appears to be better tolerated than carbamazepine. These drugs are undoubtedly valuable in the treatment of refractory epilepsy. The early clinical promise of tiagabine and remacemide are indicators that these compounds too will make the transition from interesting chemicals to useful therapeutic agents. Stiripentol and topiramate show a few more problems, but may well prove valuable as second-line therapy in refractory epilepsy. The drugs touched on in this review are merely the tip of a growing iceberg.

For an antiepileptic drug to demonstrate efficacy in refractory epilepsy is impressive. Their use as adjunctive therapy, however, is likely to exaggerate their side-effect profiles. The level playing field of double-blind, controlled trials in previously untreated patients will demonstrate the true worth of these new drugs in comparison with the much less expensive and more established compounds. Monotherapy may well prove the newer crop of anticonvulsants more benign. So far, only felbamate has been licensed for this indication, although lamotrigine is likely to be available soon as monotherapy in Europe.

The ultimate goal for everyone involved in the development of antiepileptic drugs is the discovery of effective and safe agents with well-defined mechanisms of action. We may find it a useful strategy to combine drugs that independently influence either excitatory or inhibitory processes. As time passes, the best combinations are likely to be discovered serendipitously. The use of two agents acting on the 'same side of the fence', such as vigabatrin and tiagabine, may be a logical approach in refractory single seizure types. Those with different modes of action, such as vigabatrin and lamotrigine, may be best combined for patients experiencing more than one seizure type. A narrower range of biochemical effects may lead to enhanced tolerability during such dual therapy, and 'selective' co-prescribing could be the key to improving seizure control with fewer side effects. This approach should supersede the current empiricism, and lead to the rational evolution of combination therapy for the treatment of refractory epilepsy.

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Lamotrigine

Clinical Use

John P. Leach and Martin J. Brodie

In the 1960s, some anticonvulsants were observed to give rise to abnormalities in folate metabolism and a little later folate itself was shown to be proconvulsant in rodents (17). Around that time, Reynolds and his colleagues (30) proposed that antifolate drugs would have anticonvulsant properties. Lamotrigine, a phenyltriazine compound, was born out of this suggestion after a search by Wellcome Laboratories for an appropriate folate antagonist. However, its anticonvulsant efficacy has proved far greater than its effect on folate. These properties are now known to be independent. At the time of writing, lamotrigine is licensed for use as add-on therapy in more than 20 countries worldwide and is undergoing clinical trials to ascertain its suitability for use as monotherapy.

PLACEBO-CONTROLLED TRIALS

Eleven placebo-controlled, double-blind trials (Table 1), involving almost 1,000 patients, have confirmed the efficacy of lamotrigine for partial seizures with or without secondary generalization (6,7,13, 19,22,23,32,35,37,38,40). Most were of crossover design, whereas three involved parallel groups (13, 23,37). In some of the early studies (6,7,19), unblinded observers were used to control the dose of lamotrigine, keeping the circulating concentration within a preset range. Not all these trials have as yet been published in full.

In the preliminary report by Binnie (6), ten patients with treatment-resistant partial seizures were recruited and all completed the study. There was a significant decrease in seizure counts with lamotrigine compared to placebo. Six noted a decrease in seizure frequency of at least 50% on lamotrigine, with one patient remaining seizure-free throughout the 7-day treatment period. The active drug was well tolerated; side effects occurred in three patients at concentrations above 3 mg/liter and abated following a reduction in lamotrigine dosage.

Jawad and colleagues (19) maintained trough levels of lamotrigine between 1.5–2 mg/liter. They found a significant reduction in seizure days and number of seizures among their 21 patients over 12 weeks of therapy. This was most marked after the first month. Two thirds of patients had their total seizure count more than halved. Seventy-five percent of patients with partial seizures responded to lamotrigine, compared with 44% with secondary generalization. This reduction was statistically significant for both seizure types. Lamotrigine was well tolerated, with seven adverse events documented, two of which occurred during placebo administration. Four were reported by one patient, who (unsurprisingly!) was withdrawn from the study. The most common side effects were headache, diplopia, drowsiness, and ataxia.

In Binnie's later study (7), 30 patients were given lamotrigine as add-on therapy. Although two patients reported side effects with lamotrigine, seven experienced these while taking the placebo. Only one patient was withdrawn as a result of an adverse event, a maculopapular rash. In seven patients the lamotrigine dose was reduced, mostly because of headache and dizziness. The efficacy of lamotrigine was confirmed, with 12 patients having their total seizure count reduced by more than 25% on lamotrigine compared with only four on placebo. Twenty of the 22 patients with partial sei-

TABLE 1. *Placebo-controlled trials with lamotrigine*

Reference number	Patients recruited	Patients completing	Duration of treatment (weeks)	Dose range (mg/day)	Decrease in total seizure frequency (%)	Patients with >50% decrease in total seizures	Patients with >50% decrease in partial seizures	Patients with >50% decrease in generalized seizures
6	10[a]	10	1	50–400	NA	60%	5/8 (62.5%)	5/5 (100%)
13	24[a]	21	12	75–400	59 (median)	67%	12/17 (71%)	7/15 (47%)
7	34[a]	30	12	75–200	17 (median)	7%	2/20 (10%)	2/19 (9%)
22	25[a]	23	8	75–300	23 (median)	30%	8/23 (35%)	NA
32	88[a]	NA	12	100–400	25 (median)	20%	NA	NA
35	21[a]	18	12	100–300	18 (mean)	11%	NA	NA
13	216[b]	NA	24	500	32 (median)	34%		
				300	23 (median)	20%	NA	NA
				placebo	14 (median)	NA		
37	446[b]	NA	24	NA	NA	NA	NA	NA
38	41[a]	41	12	300 (non-VPA) 150 (VPA)	24 (median)	22%	8/41 (20%)	9/32 (28%)
40	81[a]	62	18	100–400	30 (mean)	18%	12/62 (19%)	10/36 (28%)
23	191[b]	NA	24	500 (non-VPA)	36 (median)	34%		
				300 (VPA)	20 (median)	20%	NA	NA
				Placebo	8 (median)	18%		

[a] Crossover trials.
[b] Parallel groups.
NA, data not available.

zures noted an improvement in seizure numbers. This exceeded 50% in just two.

Loiseau and coworkers (22) found that over an 8-week period, 15 of 23 patients reported a reduction in total seizures while taking lamotrigine compared with placebo. Fourteen had the frequency of their partial seizures reduced, eight by more than 50%. Three patients noted vertigo and two experienced some nervousness. Seven other minor self-limiting adverse events were documented with lamotrigine, compared with four during the placebo phase. The mean trough lamotrigine concentration in patients who responded to the drug was 1.5 mg/liter, and, unusually, there was a hint of a correlation between efficacy and circulating concentration.

Risner (32) and Messenheimer et al. (23a) reported the results of a placebo-controlled, double-blind study in 98 patients recruited in seven centers across the U.S. Most patients received a lamotrigine dose of 400 mg/day. During the 14-week treatment period, the overall median seizure frequency decreased 25%, with 20% of the patients having a 50% or greater reduction in seizure frequency.

The results from Sander's study (35) were the least conclusive. Eighteen patients completed the trial. Three were withdrawn, one due to a complication of a seizure and another following an overdose of baseline anticonvulsant. The third patient developed symptoms that eventually resolved on valproate withdrawal. There was an overall reduction in seizure numbers, which was greater for secondarily generalized events in the latter stages of the lamotrigine treatment period. Partial seizures appeared unaffected. A maximum of 200 mg lamotrigine daily was prescribed if concomitant treatment included an enzyme-inducing anticonvulsant. Trough plasma levels of lamotrigine were measured. No dose-response relationship was noted, unsurprisingly perhaps, given the low concentrations. Tolerability was not a problem, with similar numbers of adverse events reported on lamotrigine and placebo. The lack of efficacy, however, was most likely due to a combination of low lamotrigine dosage and the refractory nature of the seizure disorder. All recruited patients were undergoing institutionalized care. Around 50% took at least three anticonvulsant drugs daily before entering the study, and 11 had a structural brain lesion.

Results from a large parallel-group study of 216 patients were reported by Dren et al. (13) and Matsuo et al. (23). A statistically significant decrease in seizure frequency was obtained with a lamotrigine dose of 500 mg per day compared with 300 mg daily and placebo. The beneficial effect persisted throughout the 24-week treatment period, with one third of patients taking 500 mg lamotrigine daily experiencing a 50% or greater reduction in seizures. The overall median reduction was 36% with the 500 mg dose compared with 20% for the 300 mg dose and 8% for placebo. No useful correlation was found between lamotrigine concentrations and efficacy.

Schachter and coworkers (37) conducted the largest American parallel group study on 446 patients, 112 of whom received placebo. The remaining 334 took up to 500 mg lamotrigine daily for 6 months. The efficacy data presented to date are scanty, although 65% of patients were said to have improved (unspecified criteria) on lamotrigine compared with 35% on placebo. More details are awaited. The withdrawal rate was 8% for

both groups, all because of side effects, the commonest of which was nystagmus, which occurred in 18% of patients taking lamotrigine against 11% on placebo. Adverse events requiring withdrawal of lamotrigine were dizziness (3%), blurred vision, headache, and rash (1% each).

Schapel and colleagues (38) undertook 12-week treatment with lamotrigine and matched placebo in 41 patients, all of whom completed the study. The dose of lamotrigine was dependent on concomitant anticonvulsant therapy. Twenty-six patients reported a decrease in total seizure numbers, by more than 50% in nine (22%). The median reduction in partial and secondary generalized seizures was 20% and 46% respectively. Overall, 20% and 47% of patients with partial and secondary generalized seizures had these reduced by more than 50% with lamotrigine compared with 16% on placebo. Although the fall in secondary generalized seizures did not reach statistical significance, it became more marked when patients with less than four seizures a month were excluded. Only one serious adverse event was reported, which was thought to be a complication of a seizure. The mean lamotrigine plasma concentrations were 1.95 mg/liter in enzyme-induced patients and 2.37 mg/liter in those who were not taking an inducer. A concentration-effect relationship was suggested by comparing levels in responders (>50% reduction) and nonresponders.

Smith and colleagues (40) reported on 18-week lamotrigine and placebo treatment periods. Of 81 patients recruited, 62 completed the trial. Lamotrigine doses were relatively high compared with other crossover studies—200 mg daily for those on nonenzyme-inducing drugs and 400 mg for those taking enzyme-inducing anticonvulsants. Eleven patients withdrew because of adverse events, mostly headache, diplopia, and dizziness. Eighteen of the completing patients had a modest response, reporting a reduction in total seizures of between 25% and 49% when compared with placebo. A further 11 could be regarded as responders (reduction >50%). On analysis by seizure type, 12 of 62 experienced a marked reduction in partial seizures, with 10 of 36 demonstrating a similar response in secondary generalized seizure frequency. Both observations were statistically significant. Seizure severity was ameliorated by lamotrigine. This change was thought to be independent of the positive effect of the drug on seizure frequency.

For the first time, "quality of life" factors were monitored throughout an antiepileptic drug study. Although most of the tests revealed no difference between lamotrigine and placebo, there were significant improvements in "mastery" (perceived internal control) and "happiness." Forty-two of the completing patients chose to remain on lamotrigine, some despite little change in their seizure pattern. This was interpreted by the investigators as supporting the case for psychotropic benefit with the drug.

OPEN-LABEL TRIALS AND CASE REPORTS

A number of open-label trials with lamotrigine have been carried out, and these have played a valuable role in exploring the dose requirements and identifying common side effects (31). Jawad and coworkers (20) gave 23 patients lamotrigine in addition to their usual medication for 7 days. Of the 20 who completed the study, 18 took two other drugs, the remainder receiving three. Eight of the patients (40%) had a reduction in seizure frequency exceeding 50%, whereas a similar number noted a less striking improvement.

Sander's open-label study (36) also supported efficacy for lamotrigine in patients already taking one or two antiepileptic drugs. A total of 104 patients completed 12 months of treatment with lamotrigine; 25% experienced a reduction in seizure count by more than half. Those who developed side effects did so at varying concentrations. This study did not support the concept of a useful target range of plasma lamotrigine concentrations. The drop-out rate was 15%. Of these 19 patients, 15 experienced side effects associated with lamotrigine. As in other studies, the commonest problems were headache, diplopia, drowsiness, and ataxia.

Betts (4) pooled the results of 27 similar open-label studies involving a total of 572 patients. Lamotrigine doses varied between 200–400 mg daily in induced patients and 100–200 mg in noninduced patients. The changes in seizure frequency were compared over four 12-week periods with a 3-month baseline. Of the 211 patients with secondary generalized seizures, 40% showed a substantial reduction in seizure frequency after 12 weeks of lamotrigine treatment, with 13% becoming seizure-free. The 361 patients with partial seizures did slightly less well, but 29% had their seizure numbers cut by more than half.

A report of its intravenous use in status epilepticus suggests that the drug also possesses acute anticonvulsant properties (28).

Anecdotal reports support benefit for lamotrigine in patients with primary generalized tonic–clonic seizures (34,41,44), typical and atypical absences (3), atonic (29), and myoclonic seizures (46).

EFFICACY IN CHILDREN

There are few studies with lamotrigine in children (50). Current evidence, however, supports similar efficacy in this patient population to that documented in

TABLE 2. *Percentage of patients with ≥50% seizure reduction during the first 12 weeks of lamotrigine therapy*

Seizure type	Children (n = 285)	Adult (n = 677)
Total seizures	34	32
All partial	31	30
Simple partial	14	24
Complex partial	34	31
Secondary generalized	33	42
Typical absence	53	34
Atypical absence	50	61
Myoclonic	31	31
Clonic	24	36
Tonic–clonic	30	38
Atonic	38	60

Data on file at Wellcome Laboratories.

adults (Table 2). Chaves and colleagues (11) included 36 patients aged between 5 and 15 years in a single-blind trial of lamotrigine preceded by a placebo baseline phase. Among the 31 completing patients, 12 had their seizure counts reduced by at least 50%. An increase in seizure frequency was noted in two children and in 17 there was no change.

A total of 249 children aged 2–16 years with refractory epilepsy were included in an assessment of adjuvant lamotrigine by Hosking and Spencer (18). The drug was well tolerated, with only 26 patients withdrawn due to lack of efficacy or deterioration in seizure control. Ten patients experienced a rash, all within the first few months of treatment. Of the first 36 patients to complete 12 weeks treatment, 15 had their seizure numbers cut by more than half. The best responses were seen with myoclonic jerks and absence, tonic, and atonic seizures. There was a suggestion of improved behavior in some of the children successfully treated.

In a review of 59 students attending a special residential school, further details were given regarding 12 children who had spike-wave discharges suitable for automatic monitoring (3). Six of these showed a dramatic reduction in their spike-wave events with lamotrigine treatment. In some patients this was not accompanied by reduction in overt seizures, but nevertheless conferred considerable benefit in terms of improved alertness and behavior.

A review of 120 children treated in Paris (39) reported 40% experiencing at least a 50% reduction in total seizures; 10% became seizure-free. The best results were obtained in patients with generalized seizures, including absences, Lennox-Gastaut syndrome, and other types of symptomatic generalized epilepsy. In another open study, 18 children between the ages of 5 and 11 were given lamotrigine as add-on therapy (48). Fifteen remained on the treatment for at least 24 weeks with marked or moderate overall improvement seen in eight. A further report suggests that atypical absence and complex partial seizures respond best to lamotrigine (15). A few children have been established on lamotrigine monotherapy (24).

The use of lamotrigine in Lennox-Gastaut syndrome has been investigated by three groups (25,39,45). A total of 45 affected patients have been treated. Promisingly, 33% demonstrated complete disappearance of seizures for up to 2 years of follow-up. Overall, 50% of patients reported a decrease in seizure numbers exceeding 50%. Double-blind studies with the drug in therapy-resistant Lennox-Gastaut syndrome are awaited. A preliminary report also suggests benefit with lamotrigine in children with Rett's syndrome (47).

MONOTHERAPY

Eight patients with partial epilepsy, who had completed a double-blind, placebo-controlled, add-on trial with lamotrigine had their concomitant antiepileptic medication withdrawn (2). This was successfully achieved in seven, who had a reduction or, at least, no change in seizure frequency. Only one patient was returned to polytherapy. Among the others, the incidence of side effects was much reduced. Avrutsky (1) looked at patients at the end of a 6-month lamotrigine treatment period. Of the 36 who stayed on the drug, eight had their other antiepileptic drugs discontinued.

Timmings and Richens (46) undertook a pilot trial in 17 patients with juvenile myoclonic epilepsy, all of whom were receiving sodium valproate. After a 4-week single-blind, placebo-controlled, add-on period, patients were randomized to receive either lamotrigine or valproate as monotherapy for a further 12 weeks. One patient dropped out in the add-on phase due to the onset of dizziness. Three patients withdrew during the crossover to lamotrigine, one with increased seizure frequency and the other two with rash. Otherwise, seizure control was comparable between valproate and lamotrigine monotherapy.

A multicenter, open-label trial of lamotrigine as monotherapy was conducted in patients whose seizures were uncontrolled by carbamazepine, phenytoin, or sodium valproate (10). After a 4-week lamotrigine titration phase, the patients were followed up for 12 weeks to assess the clinical response. The other antiepileptic drug was withdrawn if the addition of lamotrigine produced a decrease in seizure frequency of more than 50%. Patients on lamotrigine monotherapy were followed up for 12 additional weeks. Eighty-one percent of these patients were sustained successfully on lamotrigine monotherapy. On the negative side, there were 127 drop-outs. Thirty of these were for lack of lamotrigine efficacy and 48 for side effects severe

enough to warrant lamotrigine withdrawal, 16 of whom developed rashes (12).

Preliminary analysis has been made of an open, multicenter monotherapy trial of lamotrigine versus carbamazepine (10). Suitable patients for inclusion were aged 16–65 years. They had suffered two or more partial or secondary generalized tonic–clonic seizures during the previous 6 months with at least one within the past three months. Randomisation was blinded to lamotrigine or carbamazepine. Three parallel treatment schedules were available. Following a 6-week dosage escalation phase, patients were established on lamotrigine 100 mg or 200 mg daily or carbamazepine 600 mg daily. They were regarded as having completed the study if they had a further seizure on maintenance treatment, or if they finished the 24-week follow-up period seizure-free.

Similar numbers of patients have remained seizure-free on all three schedules. Seven receiving carbamazepine were withdrawn due to adverse events compared with one on lamotrigine. This latter patient developed a rash on the higher lamotrigine dose. Four patients treated with carbamazepine also had rashes. Nausea and tiredness, ataxia and dizziness, and abnormal menstruation were the reasons given by the other three patients for being unable to tolerate carbamazepine.

Lamotrigine is presently undergoing a program of double-blind trials as monotherapy in newly diagnosed epilepsy. More than 250 patients have completed comparative studies against carbamazepine and phenytoin and definitive reports of these important studies will be available soon.

USEFUL COMBINATIONS

The development of rational schemes for combining antiepileptic drugs may be one of the positive spin-offs of developing agents with single and specific mechanisms of action. The possibility of manipulating opposite sides of the neurotransmitter balance, involving neuronal inhibition and excitation, with, for example, vigabatrin enhancing inhibition and lamotrigine antagonizing excitation, is an exciting one. Whether this will be more effective than combining drugs that influence the same side at two different points, such as by using lamotrigine with remacemide, will become apparent on appropriate clinical testing.

The beneficial effect of combining sodium valproate and lamotrigine (26,27) was marked following failure of both drugs individually in treatment of a small number of patients with intractable typical absence and partial seizures.

Similarly, increased efficacy has been reported anecdotally when lamotrigine was tried with vigabatrin (21,42). Stolarek and colleagues (43) carried out a placebo-controlled, double-blind, crossover trial of additional lamotrigine in patients receiving vigabatrin as part of their anticonvulsant regime. As neither drug had a license for monotherapy, it was not possible to combine just lamotrigine and vigabatrin. There was a reduction by 37% in overall seizure count during the lamotrigine treatment period compared with placebo among the 20 patients completing the trial. At the highest lamotrigine dose (100 mg twice daily) nine patients reported a fall in seizure numbers exceeding 50%. Both partial and secondarily generalized seizures were reduced. The plasma lamotrigine concentrations did not correlate with clinical efficacy in this study, in common with most others. By manipulating further the therapeutic regimes, five patients were subsequently rendered seizure-free on lamotrigine and vigabatrin alone.

This favorable response was supported by two open-label studies using combinations containing vigabatrin and lamotrigine (14,33). Robinson and colleagues (33) looked at 48 patients. In the total group, there was a 54% decrease in mean monthly seizure frequency. Seventy-three percent of patients with partial seizures experienced a reduction of greater than 50%. Forty-five percent of patients with generalized seizures, consisting mainly of Lennox-Gastaut syndrome, had their seizure frequency reduced by at least half. Froscher (14) tried this combination in 12 patients with refractory epilepsy. Seven remained on the combination with good effect, with three being withdrawn due to lack of efficacy and two experiencing side effects.

INITIATION AND MAINTENANCE OF THERAPY

The lamotrigine elimination half-life is substantially prolonged in valproate-treated patients (49), an effect negated by concomitant enzyme-inducing drugs which themselves accelerate lamotrigine metabolism (8). In addition, there is good, if anecdotal, evidence that a low, slow titration schedule will reduce the likelihood of rash (12). Accordingly, the starting dose and rate of titration for lamotrigine as adjunctive therapy depends on existing treatment.

Tablets containing 25 mg, 50 mg, 100 mg, and 200 mg lamotrigine are available. Chewable tablets containing 5 mg, 25 mg, and 100 mg lamotrigine provide an alternative preparation for children and for patients who have difficulty in swallowing. These can also be dispersed in a small volume of water. Lamotrigine is usually prescribed twice daily, but a single daily dose can be used in patients taking the drug along with sodium valproate or as monotherapy. A parenteral formulation is in development.

Recommended dosing schedules for lamotrigine are

TABLE 3. *Lamotrigine dosage schedules*

1. Add-on in treated adults and adolescents		
	Valproate	Others
Weeks 1–2	25 mg every other day	50 mg daily
Weeks 3–4	25 mg daily	50 mg twice daily
Maintenance	50–100 mg twice daily	100–200 mg twice daily
2. Add-on in treated children		
	Valproate	Others
Weeks 1–2	0.2 mg/kg	2 mg/kg
Weeks 3–4	0.5 mg/kg	5 mg/kg
Maintenance	1–5 mg/kg	5–15 mg/kg
3. Monotherapy in newly diagnosed epilepsy		
	Adults	Children
Weeks 1–2	25 mg daily	0.5 mg/kg
Weeks 3–4	25 mg twice daily	1 mg/kg
Maintenance	50–100 mg twice daily	2–8 mg/kg

Higher doses can be tried if seizures persist and the patient is tolerating the drug without complaint.

outlined in Table 3. Higher doses can be tried if the drug is well tolerated. Although no definite evidence of teratogenicity has accumulated, caution should be employed when using lamotrigine in patients with child-bearing potential. The drug should only be used when, in the opinion of the attending physician, the likely benefit outweighs the potential risks.

Like other antiepileptic drugs, lamotrigine appears to exacerbate seizures in a small number of patients in whom it should be rapidly withdrawn. Reducing the dose by 50–100 mg weekly seems a reasonable policy (9). Because the drug is metabolized in the liver, it is sensible to avoid its use in patients with severe hepatic impairment. There is little formal experience with lamotrigine in babies, in the elderly, or in patients with renal failure.

At present, it appears that no clinical benefit can be obtained by monitoring plasma lamotrigine levels, because the concentrations required to elicit an adequate response vary so much between individuals. There is no evidence either that subjective side effects are more likely to occur in patients with higher concentrations (5). Indeed, some patients will derive benefit from as much as a gram of lamotrigine daily without complaint, whereas others will develop headache or nausea and vomiting with doses as low as 100 mg daily. Further studies exploring the concentration-effect-toxicity relationship with the drug are warranted.

INDICATIONS

Lamotrigine is licensed in more than 20 countries worldwide as add-on therapy for the treatment of refractory partial and secondarily generalized seizures. Long-term studies up to 3 years have revealed no evidence of tolerance (16). Anecdotal reports suggest efficacy for the drug across the range of idiopathic generalized epilepsies. Lamotrigine might, therefore, be tried in patients with refractory absence, tonic–clonic, myoclonic, tonic, and clonic seizures. Its use in combination with sodium valproate and vigabatrin shows particular promise.

Results from comparative double-blind trials in newly diagnosed patients with partial and primary generalized tonic–clonic seizures are imminent. However, lamotrigine can be useful in those few patients unable to tolerate, for one reason or other, the first-line agents. Its efficacy in the elderly is currently being evaluated in a double-blind comparative trial against carbamazepine and its promise in primary generalized tonic–clonic epilepsy is being formally tested against sodium valproate. Double-blind, placebo-controlled trials in children are also under way.

CONCLUSIONS

Lamotrigine is a potentially important addition to the ranks of available anticonvulsants. It has proven efficacy in adults and adolescents for partial and secondary generalized seizures. Anecdotal reports also support particular efficacy in the idiopathic generalized epilepsies. Its broad spectrum of activity is wide enough to justify using it in patients with more than one seizure type. The next important step is for lamotrigine to gain acceptance as a first-line drug for use as monotherapy in patients with newly diagnosed epilepsy. Data are still required in children, in the elderly, and in the idiopathic generalized epilepsies.

The possibility of rational duotherapy with established and novel anticonvulsants provides an exciting prospect for future clinical research. Combinations of lamotrigine with sodium valproate and vigabatrin appear particularly promising. This will hopefully be confirmed in due course in formal double-blind trials. Studies looking at lamotrigine along with other drugs that affect excitatory amino acid neurotransmission are also awaited with interest.

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CASE REPORT

Deliberate overdose with the novel anticonvulsant tiagabine

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Tiagabine is a novel antiepileptic drug which acts by decreasing gamma aminobutyric acid uptake in astrocytes and neurones. Here the first case of deliberate overdose with this compound in a patient on concomitant phenytoin is reported. On admission to hospital his conscious level deteriorated to grade III coma. No changes in the electrocardiogram were noted. Recovery from the initial effects was rapid, and there were no sequelae. Plasma levels of tiagabine (3.1 μg/ml) 4 hours after ingestion were 30 times higher than at typical steady state during therapeutic dosing. The effects of poisoning with current first-line antiepileptic drugs are reviewed. The newer agents, particularly those with greater biochemical specificity, may be safer in overdose than the more established anticonvulsants.

Key words: tiagabine; overdose; epilepsy; self poisoning.

INTRODUCTION

Epileptic patients are at greater risk of self poisoning than other patient groups[1]. Safety in overdose should, therefore, be an important consideration when choosing an antiepileptic drug. Tiagabine is a novel anticonvulsant, which is currently undergoing phase III trials worldwide[2]. It is a nipecotic acid derivative with a lipophilic anchor, which allows it to cross the blood–brain barrier. Tiagabine acts by blocking the re-uptake of the inhibitory neurotransmitter gamma animobutyric acid (GABA) into glial cells and neurones[3]. The resultant increase in synaptic GABA concentration is thought to be responsible for its anticonvulsant action[4]. After oral administration, it is rapidly absorbed and metabolized in the liver with a half-life of between 4 and 13 hours. Although sedation has been reported, preliminary data suggest that tiagabine does not impair cognition at therapeutic dosage[5]. We report the first case of deliberate overdose with tiagabine.

CASE REPORT

DR is a 30-year-old man who has suffered from complex partial and secondary generalized seizures of unknown aetiology since the age of three. Surface electroencephalography demonstrated recurrent focal epileptiform discharge in the right fronto-temporal area. Computerized tomography of the brain was normal. Prior to this episode he had been taking tiagabine (64 mg daily) in addition to his usual dose of phenytoin (200 mg daily) with substantial benefit under an open protocol for the previous 9 months.

Following an argument with his girlfriend, the patient took 320 mg of tiagabine together with 400 mg phenytoin. One hour later, he became drowsy and he was admitted to the local hospital. His conscious level deteriorated rapidly, reaching grade III coma overnight. Routine biochemistry, liver function tests and full blood counts were all normal. No active intervention was undertaken and the patient recovered fully. No neurological deficit could be demonstrated 12 hours after admission. Serial electrocardiograms showed no

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abnormality through his hospital stay. Tiagabine and phenytoin plasms concentrations 4 hours after ingestion were 3.1 μg/ml and 22 μg/ml, respectively. Psychiatric review confirmed that this was an impulsive overdose with no suicidal intent. He was discharged home after 48 hours' observation.

DISCUSSION

The current first-line antiepileptic drugs are all potentially fatal in overdose[1], perhaps a consequence of their multiple, non-selective mechanisms of action[6]. In a three-year prospective survey of overdoses of non-barbiturate anticonvulsants reported to the UK National Poisons Service, carbamazepine (48%), phenytoin (33%) and sodium valproate were the most frequently ingested[7], perhaps not surprisingly as they are regarded as the antiepileptic drugs of first choice[8].

The initial features of carbamazepine poisoning are those of cerebellar dysfunction. As the concentration increases, central nervous system depression becomes dominant, progressing to respiratory depression in severe cases[9]. Anticholinergic features appear, presumably as a consequence of carbamazepine's structural similarity to the tricyclic antidepressants. An increase in the frequency of convulsions has been noted, perhaps related to the production of hyponatraemia[10]. The risk of cardiac arrhythmias necessitates ECG monitoring for at least 24 hours[11].

The saturation pharmacokinetics of phenytoin means that the ingestion of even a small dose can produce a substantial increase in circulating concentration[12]. The slow rate of hepatic metabolism can make the recovery period as long as a week[13]. The initial features of acute phenytoin toxicity are cerebellar dysfunction including nystagmus, incoordination, dysarthria and ataxia[14]. Depression of the central nervous system (CNS) is common, often progressing to coma. This may be accompanied by hypotension and respiratory depression.

With sodium valproate, central nervous system depression is predominant, although often benign[15]. Coma, however, can occur after ingestion of more than 20 mg/kg body weight. Seizures[16], respiratory failure, bone marrow suppression[17] and metabolic adverse effects such as acidosis and hypocalcaemia can all occur[18]. Fatalities, although uncommon, have been reported[17,18].

Our patient was receiving treatment with phenytoin and tiagabine prior to the event. The tiagabine concentration four hours after ingestion was 30 times higher than at typical steady state following therapeutic dosage (Dr LC Lassen, pers. comm.). This is equivalent to around 300 μmol/litre of carbamazepine or 450 μmol/litre of phenytoin. At that time the patient was in light coma. The phenytoin concentration just exceeded the target range, and so was unlikely to contribute to the clinical picture. Induction of tiagabine's metabolism by phenytoin might have contributed to the patient's rapid recovery[19].

There are no published cases of self poisoning with the GABA transaminase inhibitor vigabatrin, but current information suggests that the only adverse effect is transient drowsiness (Dr J Mumford, pers. comm.). There has been one report of lamotrigine overdose, in which neurological toxicity was a feature[20]. This is the first report of tiagabine taken in overdose in man, and the rapid, uneventful recovery is, we believe, reassuring for this vulnerable patient population. One of the individual benefits of the newer antiepileptic drugs may be that their increased biochemical specificity augments existing physiological processes, even in overdose, rather than producing a 'blunderbuss' deleterious effect on a range of cerebral functions and peripheral tissues. Consequently, anticonvulsants with defined mechanisms of action may be safer in overdose than the more established agents.

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Enclosure (iv)

DETERMINATION OF GABAPENTIN IN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Keywords: Gabapentin, high-performance liquid chromatography

Running title: Gabapentin by HPLC

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ABSTRACT

A rapid and simple method for determination of the novel antiepileptic compound gabapentin (1-(aminomethyl)-cyclohexaneacetic acid) in plasma is described. Blank human plasma was spiked with gabapentin (1.0 - 10.0 μg/ml) and internal standard (1-(aminomethyl)-cycloheptaneacetic acid; 5.0 μg/ml). Individual samples were treated with 2M perchloric acid, centrifuged and then derivatised with *o*-phthalaldehyde-3-mercaptopropionic acid. Separation was achieved on a Beckman Ultrasphere 5μ reversed phase column with mobile phase consisting of 0.33 M acetate buffer (pH = 3.7; containing 100 mg/l EDTA) / methanol / acetonitrile (40:30:30). Eluants were monitored by fluorescence spectroscopy with excitation and emission wavelengths of 330 and 440 nm respectively. The calibration curve for gabapentin in plasma was linear ($r = 0.9997$) over the concentration range 1.0 - 10.0 μg/ml. Recovery was seen to be ≥ 90 %. The inter- and intra-assay variations for three different gabapentin concentrations were ≤ 10 % throughout. The lower limit of quantitation was found to be 0.25 μg/ml. Chromatography was unaffected by a range of commonly employed antiepileptic drugs or selected amino acids.

INTRODUCTION

Gabapentin (GBP) is a novel antiepileptic drug (AED) that has recently been approved in the UK and USA for the treatment of partial seizures. It is a hydrophilic analogue of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and was designed to act as a $GABA_A$ receptor agonist that could freely cross the blood-brain barrier [1].

Despite its structural similarity to GABA (figure 1), GBP has demonstrated only limited effects on the GABAergic system. It is devoid of effect on GABA-mediated ion conductances, GABA receptor binding and GABA metabolism [2,3]. Although the mechanism of GBP action remains to be fully characterised, substantial evidence now suggests that it may interact specifically with a plasma membrane site proposed to be the system L-amino acid transporter [4,5].

GBP has an experimental anticonvulsant profile similar to that of valproic acid [6]. It is effective against tonic seizures induced by a variety of chemoconvulsants [6] and is also active in the maximal electroshock test [6] and several rodent models of genetic reflex epilepsy [7]. Clinically, the drug has demonstrated efficacy against both partial and generalised tonic-clonic seizures [8].

GBP is rapidly absorbed and exhibits a dose-dependent bioavailability as a result of a saturable uptake process [9]. Maximum concentrations occur 2 - 3 hours after administration and the elimination half-life is approximately 5 - 7 hours [10]. There is no

significant binding to plasma proteins, and the drug is excreted unchanged in the urine with a clearance rate equivalent to that for creatinine [11]. Despite extensive pharmacokinetic investigations, and the report that GBP is free of important drug interactions [9], the requirement for a reliable, routine laboratory assay of GBP concentration remains, particularly for those who advocate therapeutic monitoring of this drug in the treatment of epilepsy [12].

There are currently two published methods for the laboratory measurement of GBP, a high-performance liquid chromatographic (HPLC) assay [13] and a gas chromatographic (GC) assay [14]. The routine HPLC method, a modified version of a HPLC assay for GABA, would appear, however, to have several significant drawbacks. These include a multistep derivatisation involving 2,4,6-trinitrobenzenesulphonic acid (TNBS) followed by an extraction into toluene, and a methodology apparently incompatible with the use of modern, automated HPLC systems.

We have developed an automatable, one step derivatisation method for the determination of GBP in plasma by HPLC with fluorimetric detection. This rapid and reliable assay, which obviates the requirement for hazardous chemicals such as TNBS and toluene, is a modification of the method of Durkin and colleagues [15] for analysis of neurotransmitter amino acids in brain.

EXPERIMENTAL

Reagents

GBP (1-(aminomethyl)-cyclohexaneacetic acid) and the internal standard (1-(aminomethyl)-cycloheptaneacetic acid) were supplied by Parke-Davis Pharmaceuticals Resesarch Division, Ann Arbor, Michigan, USA. Methanol and acetonitrile (HPLC grade) were from Rathburn Chemicals, Walkerburn, Scotland. All other chemicals (reagent grade) were obtained from Sigma Chemical Co, Poole, Dorset, England.

Standards

Stock solutions of GBP (1 mg/ml) and internal standard (1 mg/ml) were prepared in de-ionised water and stored at -20 ^{0}C for up to 7 days. Working standard solutions of GBP (10 - 100 µg/ml) and internal standard (50 µg/ml) were prepared daily in de-ionised water. The derivatisation reagent, *o*-phthalaldehyde-3-mercaptopropionic acid (OPA-MPA), was prepared weekly by dissolving 50 mg OPA in 4.5 ml of methanol and adding 0.5 ml borate buffer and 50 µl 3-MPA. The borate buffer was prepared on a weekly basis by adjusting 0.5 M boric acid to pH 9.5 with 1 N NaOH.

Sample preparation

GBP standards were prepared by the addition of 50 µl of the appropriate working standard (10 - 100 µg/ml) and 50 µl of working internal standard to 0.4 ml blank human plasma. Samples for analysis were prepared by adding 50 µl of working internal standard to 0.45 ml unknown plasma. Pooled plasma, spiked at high (5.0 µg/ml), medium (2.5

µg/ml), and low (0.5 µg/ml) GBP concentrations was used to determine intra- and inter-assay variations.

Derivatisation

A 200 µl volume of 2 M perchloric acid was added to each standard and sample before vortex mixing for 10 seconds and centrifuging for 3 minutes at 15,000g at room temperature. A 50 µl aliquot of the resulting supernatant was reacted with 200 µl of methanol, 200 µl of 0.5 M borate buffer (pH = 9.5) and 50 µl OPA-MPA solution. The reaction mixture was allowed to stand at room temperature for 5 minutes prior to injection of 20 µl onto the column. The derivatised GBP and internal standard were found to be stable for between 4 and 12 minutes prior to injection.

High Performance Liquid Chromatography

Chromatography was carried out at room temperature on a Beckman Ultrasphere octadecyl silane (ODS) 5µ reversed phase column (250 x 4.6 mm; 80Å pore; Beckman Instruments Inc., Fullerton, California, USA). The chromatography system consisted of a Waters 6000A pump (Waters / Millipore UK, Harrow, Middlesex, England), a Shimadzu SIL-9A auto-injector (Dyson Instruments Ltd., Houghton-le-Spring, Tyne and Wear, England) and a Perkin-Elmer LS5 fluorescence spectrophotometer (Perkin-Elmer, Beaconsfield, Buckinghamshire, England). The excitation and emission wavelengths were 330 and 440 nm respectively with slitwidths set at 15 and 20 nm respectively. The mobile phase consisted of 0.33 M acetate buffer (containing 100 mg/l EDTA) / methanol /

acetonitrile (40:30:30). The acetate buffer was prepared by diluting 7.5 ml glacial acetic acid (approx 17.4 M) to 400ml with water, adding 40 mg EDTA and adjusting the pH to 3.7 with 3 N NaOH. Flow rates were 1.5 ml/min throughout.

Calculations

Chromatograms were recorded and integrated on a Jones Chromatography JCL6000 chromatography data system (Crawford Scientific, Strathaven, Scotland). GBP concentrations were determined by comparison of peak height ratios of analyte to internal standard, quantified in relation to volume, and expressed as μg/ml. Pearson's product moment correlation coefficient is quoted.

RESULTS

GBP and the internal standard were well resolved from one another and the solvent front (figure 2). The calibration line (slope = 296.757, y-intercept = 39.44) was shown to be linear from 1.0 - 10.0 μg/ml (n = 6; r = 0.9997). The intra-assay variations at 0.5, 2.5, and 5.0 μg/ml were 4.1, 2.2, and 3.8 % respectively. The inter-assay variations for the same samples were 10.0, 2.0, and 2.6 % respectively. Recoveries were shown to be ≥ 90 % throughout.

There were no interfering peaks from any of the following other AEDs:- phenytoin, carbamazepine, sodium valproate, phenobarbital, primidone, clobazam, clonazepam, lamotrigine, vigabatrin, oxcarbazepine, felbamate, tiagabine or remacemide.

Similarly, there were no interfering peaks from any of the following amino acids:- L-alanine, L-arginine, L-aspartic acid, L-cystine, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine or L-valine.

Typical GBP chromatograms are illustrated in figure 2. Samples were taken from a patient currently undergoing a double blind, crossover trial with GBP and placebo. One sample was taken from each treatment arm of this study where the daily GBP dose was 2400 mg.

DISCUSSION

This method employed pre-column derivatisation and fluorimetric detection for the quantitation of GBP concentrations in plasma. It facilitated clear detection and resolution of the drug, and its appropriate internal standard, with intra- and inter-assay variations of an acceptable degree. Chromatography was unaffected by other commonly employed AEDs or a variety of endogenous amino acids.

In contrast to previously published assays [13], the methodology proved to be both rapid and simple, obviating several complicated steps including the requirement for pH adjustment of a relatively small volume. Another important advantage of this method over those previously reported [13] was a reduced requirement for the use of hazardous chemicals such as toluene and TNBS. Unlike its predecessors, this method also proved compatible with modern HPLC systems which facilitate automated pre-column

derivatisation, thus reducing the number of technical-hours required to analyse a given number of samples.

One potential disadvantage of this new assay was an apparent reduction in sensitivity, with the lower limit of quantitation observed here being 0.25 μg/ml compared to 10 ng/ml previously reported by Hengy and Kölle [13]. However, in our clinical practise the expected plasma concentration on the least effective dose (1200 mg daily) exceeds 2 μg/ml. While saturable absorption must be taken into account, this daily dose of GBP is relatively low when compared to those of up to 6400 mg now being administered to patients with refractory epilepsy. Thus, it would appear that, with the exception of the most sensitive pharmacokinetic requirements, the new assay is more than adequate for the routine analysis of plasma GBP concentrations in the epilepsy clinic.

In conclusion, the method reported above represents a significant advance in the laboratory analysis of the novel antiepileptic drug GBP. In comparison to previously published methods [13], this HPLC assay is rapid, simple, safe and readily automatable. In addition, it appears to possess a sensitivity more than adequate for the routine monitoring of GBP concentrations in patients with intractable epilepsy.

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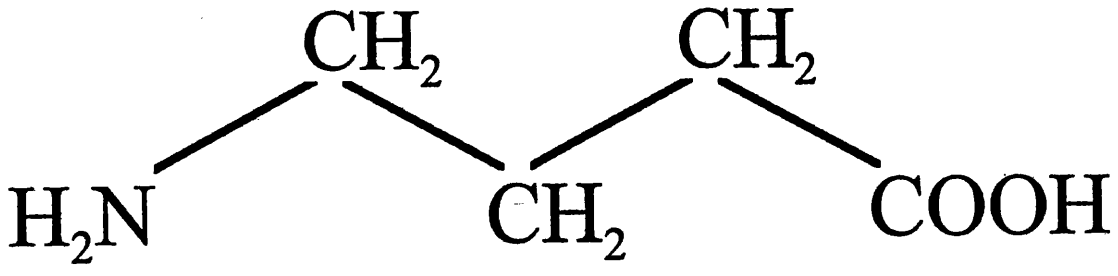
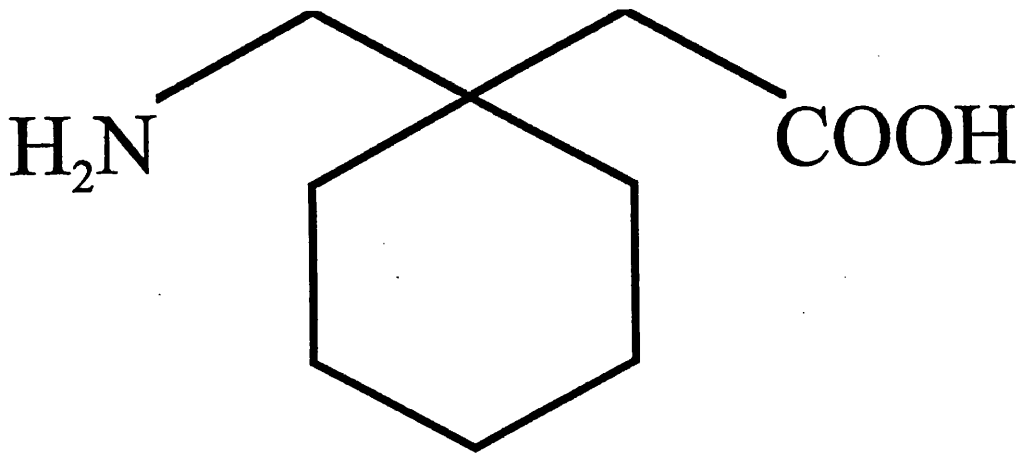
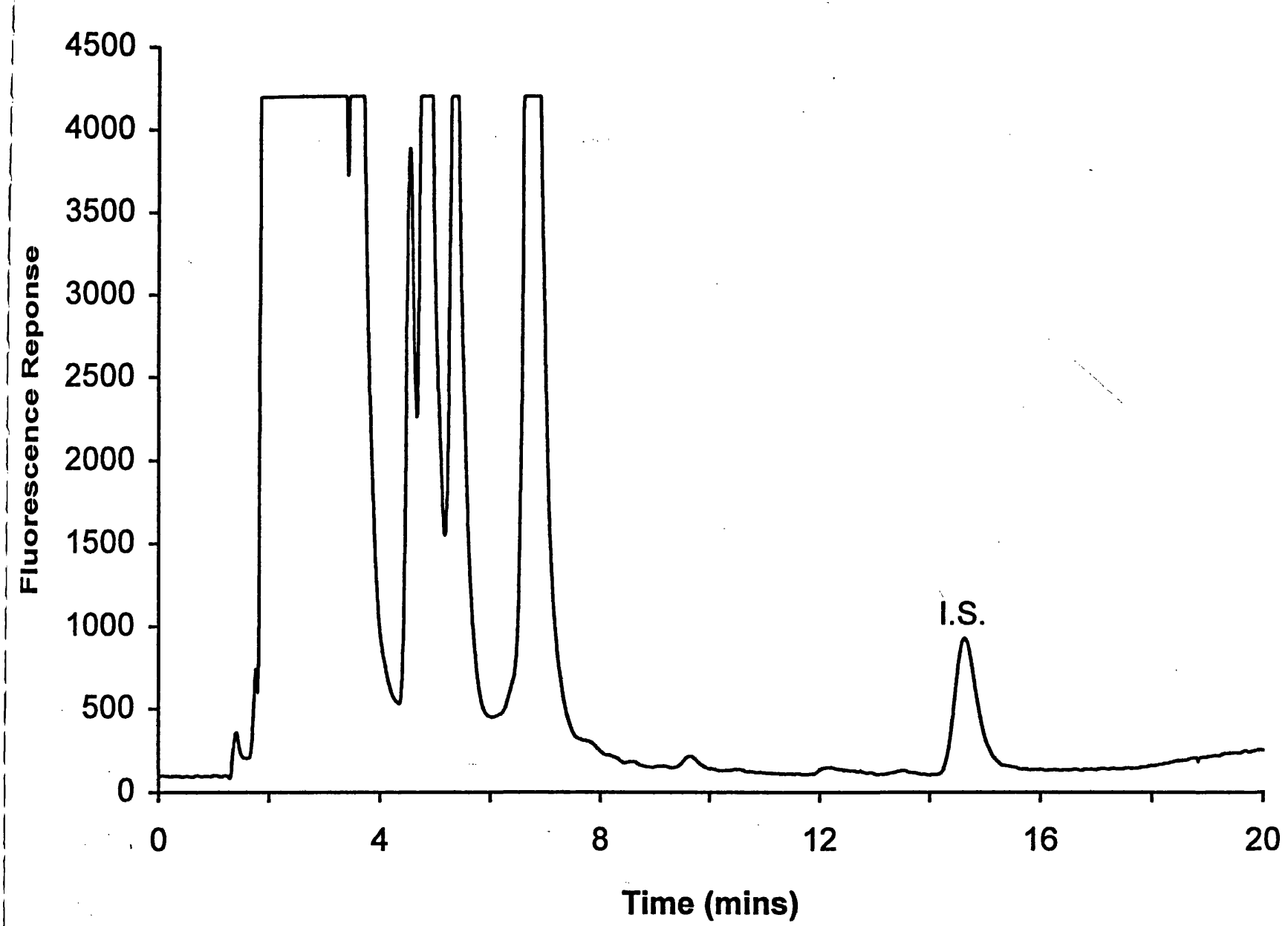


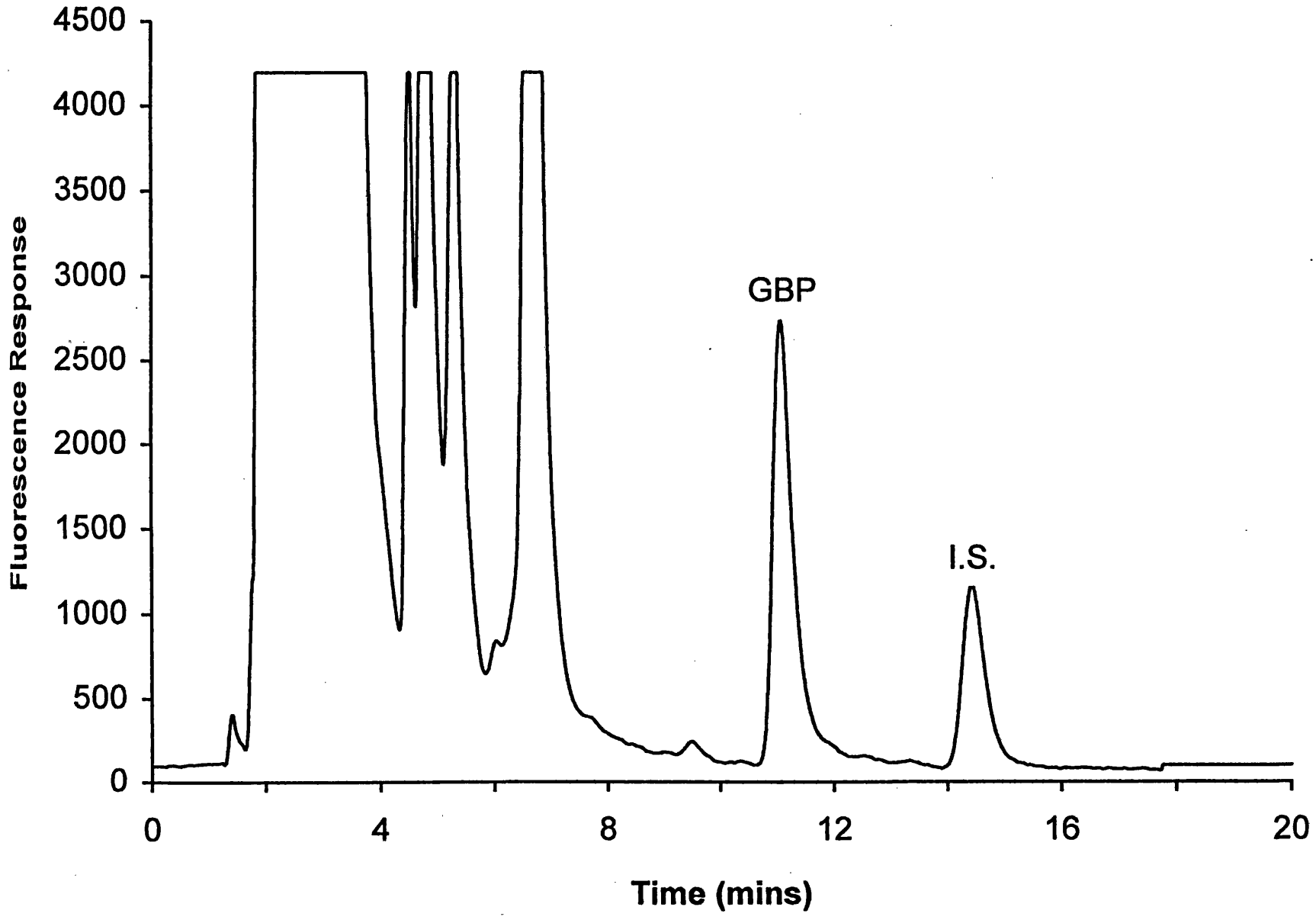
γ-AMINOBUTYRIC ACID (GABA)



GABAPENTIN

Enclosure (iv)





LEGENDS TO FIGURES

FIGURE 1 : Comparison of the chemical structures of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and the novel antiepileptic compound gabapentin (GBP).

FIGURE 2 : Typical chromatograms highlighting gabapentin (GBP) and internal standard (I.S.) peaks in one patient sample from each phase of a double blind, crossover trial of GBP and placebo. Upper chromatogram represents the placebo phase and the lower chromatogram the active phase. Daily GBP dose was 2400 mg.

Enclosure (v)
SEIZURE - In press

EFFECTS OF TIAGABINE AND VIGABATRIN ON GABA UPTAKE INTO PRIMARY CULTURES OF RAT CORTICAL ASTROCYTES

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ABSTRACT

Tiagabine (TGB) and vigabatrin (VGB) are two novel anticonvulsant compounds reported to exert their pharmacological effects via an action on the γ-aminobutyric acid (GABA) system. We have investigated the effects of acute exposure of these drugs on the uptake of GABA into rat cortical astrocytes in primary culture. Astrocytes were prepared from the cerebral cortices of one day-old rat pups by a mechanical dissociation technique and were assayed for GABA uptake activity after 21 days in culture. TGB (100 - 300 nM) and VGB (100 μM) reduced GABA uptake when compared to control at 4 hours post-exposure. GABA uptake was also reduced following 8 and 24 hour exposures to 200 nM TGB. A combination of TGB (200 nM) and VGB (100 μM) treatments reduced GABA uptake when compared to both control and VGB treated cultures. These results support the efficacy of TGB as a GABA uptake inhibitor and suggest that VGB may also exert an effect by this mechanism.

Keywords:- Tiagabine, vigabatrin, GABA uptake, cortical astrocytes, cell culture.

INTRODUCTION

Epilepsy is one of the most common neurological disorders, affecting an estimated 50 million persons world-wide [1]. The majority of the epileptic population can be adequately controlled with existing antiepileptic drugs (AEDs), although 20% of patients remain resistant to currently available treatment [2]. Recent additions to the clinician's armamentarium have, however, improved the pharmacological treatment of epilepsy, particularly in terms of side effect profiles [3].

Two such novel compounds are vigabatrin (VGB) and tiagabine (TGB). Both drugs have been reported to exert their anticonvulsant actions via specific effects on the γ-aminobutyric acid (GABA) system, VGB by an irreversible inhibition of the enzyme GABA-transaminase (GABA-T) and TGB by blockade of neuronal and glial GABA uptake [4,5].

While experimental evidence supports a single mechanism of action for TGB [6], a variety of reports would suggest otherwise for VGB. The diverse range of experimental anticonvulsant profiles exhibited by a variety of neuroactive compounds, all of which are proposed to act as inhibitors of brain GABA-T [7], might suggest the contribution of secondary mechanisms of action. Similarly, Bernasconi and colleagues [8] demonstrated that the anticonvulsant effects of VGB in animal seizure models are not related to the time of maximal GABA-T inhibition. Perhaps the most pertinent observation, however, is one of rebound seizures immediately upon clinical withdrawal of the drug [9]. Such an effect would be inconsistent with a compound which irreversibly inhibits an enzyme in the brain. As a result of these experimental and clinical observations with VGB, we have investigated its action on GABA uptake into rat cortical astrocytes in primary culture and compared these effects to those obtained with TGB.

MATERIALS

One day-old rat pups were obtained from a breeding colony of Sprague Dawley rats housed at the Joint Animal Facility, University of Glasgow. Dulbecco's modified Eagle medium (DMEM), horse serum (HS), L-glutamine, penicillin and streptomycin were all obtained from Gibco BRL (Paisley, UK). All other chemicals (reagent grade) were obtained from the Sigma Chemical Company (Poole, UK). Radiolabelled GABA (γ-[^{14}C(U)]-aminobutyric acid) was obtained from NEN Research Products (Stevenage, UK). VGB (D,L-4-aminohex-5-enoic acid) and TGB ((R-)-(-)-1-[4,4-Bis(3-methyl-2-thienyl)-3-butenyl]-3-piperidine-carboxylic acid, hydrochloride) were obtained from Marion Merrell Dow (Winnersh, UK) and Novo Nordisk A/S (Bagsvaerd, Denmark) respectively.

METHODS

Primary culture of cerebral cortical astrocytes

This method was devised from modifications of the methods of Larsson and co-workers [10] and Bender and Hertz [11]. The cerebral cortices of one day-old rat pups were removed under aseptic conditions and cleared of attached olfactory bulbs, basal ganglia, hippocampal formations and meningeal membranes. The dissected neopallia were then cut into small cubes (0.5 mm^3) by two passes (at 90°) in a McIlwain tissue chopper (Mickle Laboratory Engineering Company Ltd, Gomshall, UK). The chopped tissue was transferred to a sterile glass filter (80 μm nylon mesh; Lockertex Ltd., Warrington, UK) and the filtrate collected in a sterile beaker. The chopped material was washed through the filter with culture medium (DMEM supplemented with 20% (v/v) HS, 2.5 mM L-glutamine, 100 I.U./ml penicillin, and 100 μg/ml streptomycin) to give a final volume of 3 ml per brain. The filtrate was then passed through a sterile needle (BD Microlance 21G 0.8 x 40) three times. The volume of the resulting

suspension was adjusted with culture medium to allow a 3 ml aliquot per culture dish in a ratio of 1 brain to 3 dishes. A 3 ml volume of the final cell suspension was plated onto 60 x 15 mm Falcon Primaria culture dishes (A+J Beveridge, Edinburgh, UK). The cultures were maintained at 37^{0}C in an environment of 95% air / 5% CO_2 with a humidity of $\geq$ 90%. The culture medium (3 ml) was replaced every 3 - 4 days throughout. The HS concentration was reduced to 10% at the first medium change with a final reduction to 5% at the second change. After 14 days in culture the medium was supplemented with 0.25 mM 3′5′-dibutyryl cyclic adenosine monophosphate (cAMP) to induce cell differentiation. Penicillin and streptomycin were omitted from the medium at this stage following reports that penicillin may interfere with GABAergic function [12]. The cultures were employed for the study of GABA uptake 7 days after supplementation with cAMP.

[^{14}C]-GABA uptake into cultured astrocytes

This method was devised from modifications of the methods of Larsson and co-workers [10] and Yu and colleagues [13]. A standard balanced salt solution (BSS) was used throughout the investigations of [^{14}C]-GABA uptake. Its composition was as follows: 136 mM NaCl, 5 mM KCl, 0.8 mM $MgSO_4$, 2.6 mM $NaHCO_3$, 0.4 mM KH_2PO_4, 0.34 mM Na_2HPO_4, 1.3 mM $CaCl_2$, 5.6 mM D-glucose and 15 mM HEPES. The solution was adjusted to pH 7.4 with 1 M NaOH and stored, at 4^{0}C, for up to 1 week. BSS was warmed to 37^{0}C prior to use. Cultures for investigation were removed from the incubator and the existing medium aspirated. Cultures were washed twice (2 x 2 ml) with BSS before being returned to the incubator in a further volume of BSS (3 ml) for an equilibration period of 20 minutes. The pre-washed cultures were then removed from the incubator and the existing BSS aspirated. This solution was replaced by BSS (2 ml) containing the drug concentrations appropriate to the individual experiment.

Control plates received BSS alone. All culture plates were returned to the incubator for a further incubation period (1 - 24 hours). After the incubation period, a further 1 ml of BSS (with appropriate control / drug treatment) containing 150 μM [^{14}C]-GABA (specific activity = 1 mCi/mmol) was added to each plate. Incubation (37^{0}C) was allowed to continue for 5 minutes before the cultures were washed with 5 volumes (2 ml) of BSS. Cells were removed from the plates by scraping in 1 M NaOH (1 ml). Aliquots were taken for protein determination by the BIORAD method and liquid scintillation counting in 6 ml of Picofluor 40 scintillation fluid (Canberra Packard, Pangbourne, UK). A Canberra Packard 2000CA TRI-CARB liquid scintillation counter (Pangbourne, UK) was employed to analyse GABA uptake in individual cultures in comparison to the dpm of standard solutions containing known amounts of radioligand. Results were quantified by the relation of GABA uptake to the protein concentration and expressed as pmol/min/mg protein in individual cultures.

Determination of protein concentration

Protein concentrations were determined by the sensitive BIORAD method which relies on the colour change of a dye (Coomassie Brilliant Blue G-250). Standards were prepared over the range 5 - 20 μg/ml bovine serum albumin and samples of unknown protein concentration were also diluted into this range. BIORAD dye reagent was diluted 1:1 with water and added to standards and samples alike. Tubes were mixed and incubated at room temperature for 5 minutes and then read at 595 nm in a spectrophotometer (MR5000, Dynatech Ltd., Guernsey). Results were corrected for dilution and expressed in mg/ml.

EXPERIMENTAL PROTOCOL

The following studies were designed to investigate the dose- and time-dependent effects of both TGB and VGB on GABA uptake into primary cultures of rat cortical astrocytes. Individual studies employed control (untreated) groups and between 12 and 20 plates per group.

Study 1:- The effects of TGB dose (50 - 500 nM) on GABA uptake at 4 hours post-treatment.

Study 2:- The effects of VGB dose (1 - 500 μM) on GABA uptake at 4 hours post-treatment.

Study 3:- The effects of 200nM TGB on GABA uptake at 1 - 24 hours post-treatment.

Study 4:- The effects of 100μM VGB on GABA uptake at 1 - 24 hours post-treatment.

Study 5:- The effects of 200nM TGB and 100μM VGB, alone and in combination, on GABA uptake at 4 hours post-treatment.

STATISTICAL METHODS

Statistical analysis was performed using MINITAB for Windows statistical package (Version 10.1) on a Viglen 4DX266 microcomputer. Results were expressed as the mean percentage of mean control values for each group ± the standard error of the mean (SEM). In those experiments evaluating dose and time related drug actions (Study nos. 1 - 4), results were compared to control values by one-way analysis of variance with Dunnett correction for multiple comparisons. In the combination experiment (Study no. 5), results were compared by two sample t-test.

RESULTS

Study 1:- TGB (100 - 300 nM) significantly reduced GABA uptake into primary cultures of rat cortical astrocytes following a four hour exposure (figure 1). All other doses of TGB were without effect.

Study 2:- VGB (100 and 250 μM) significantly reduced GABA uptake into primary cultures of rat cortical astrocytes following a four hour exposure (figure 2). All other doses of VGB were without effect.

Study 3:- TGB (200 nM) significantly reduced GABA uptake into primary cultures of rat cortical astrocytes at 4, 8 and 24 hours post-exposure (figure 3). TGB was without effect at all other time points investigated.

Study 4:- VGB (100 μM) was without effect on GABA uptake into primary cultures of rat cortical astrocytes at all of the time points investigated (figure 4).

Study 5 :- TGB (200 nM), VGB (100 μM) and combination treatments all significantly reduced GABA into primary cultures of rat cortical astrocytes when compared to control (figure 5).

DISCUSSION

The aims of these studies were to investigate the effects of TGB and VGB, alone and in combination, on the uptake of [^{14}C]-GABA into rat cortical astrocytes in primary culture.

These drugs have previously been proposed to exert their pharmacological actions via single and specific effects on the GABAergic system [4,5].

TGB belongs to a new class of AEDs, derived from nipecotic acid and believed to exert their anticonvulsant action by blockade of GABA uptake into neurones and glial cells [6]. Recent evidence has suggested that GABA uptake is mediated by 4 distinct transporter proteins [14] and that TGB is predominantly active at the transporter termed "GAT-1" and weakly active at "GAT-3". TGB is effective against audiogenic seizures in DBA/2 mice [5], the motor manifestations of amygdaloid kindled seizures [15] and the tonic and clonic components of pentylenetetrazol-induced seizures in both rats and mice [5]. It has also been proposed to have efficacy against tonic seizures induced by maximal electroshock [16]. TGB is currently undergoing phase III clinical trial for the treatment of epilepsy and initial reports suggest that the drug is active against both partial and secondary generalised seizures [3].

In these studies, TGB reduced GABA uptake into rat cortical astrocytes in a dose-specific manner. Its concentration - effect profile appeared to be U-shaped, however, making determination of an IC_{50} impossible. A similar dose-related pattern has been reported with TGB in whole animal seizure models [5]. The reason for this phenomenon remains unknown, and although higher drug doses are possibly cytotoxic, no parallel reduction in the protein content of cultures was observed in the above studies. Another surprising observation from these studies was the latency to onset of TGB action on GABA uptake, with the drug only being active after at least 4 hours exposure. This would not appear to concur with the rapid onset of TGB action in whole animal seizure models following parenteral administration [5]. One might speculate that direct application of TGB to the cell surface should result in

immediate effect. Further studies employing a wider range of time intervals from 0 to 4 hours are required in an attempt to clarify this apparent discrepancy.

VGB was the first drug to enter regular clinical use, having been designed specifically for the treatment of epilepsy [17]. It has been proposed to exert its anticonvulsant effects by an irreversible inhibition of GABA-T [4], the enzyme responsible for the metabolic degradation of the inhibitory neurotransmitter GABA. VGB has demonstrated efficacy against a range of experimental seizures including those induced by picrotoxin [8] and amygdaloid kindling [18] and is also effective in genetic models of reflex epilepsy [19].

At concentrations of 100 and 250 μM, VGB significantly blocked astrocytic GABA uptake. These concentrations are close to that reported as the IC_{50} for inhibition of GABA-T in the same cell type [20]. This previously unreported mechanism of VGB action might help to explain the diverse range of experimental anticonvulsant profiles exhibited by a variety of GABA-T inhibitors [7]. It may also underlie the lack of relationship between the time of maximal GABA-T inhibition and the anticonvulsant effects of VGB in animal seizure models [8] and the observation of rebound seizures immediately upon clinical withdrawal of the drug [9].

If one considers the structural similarity between VGB and GABA, it is possible to speculate that the blockade of GABA uptake afforded by VGB may be the result of a simple competitive reaction between the two molecules at one or more of the 4 reported GABA uptake carriers [14]. Although previous studies have suggested that VGB is not a substrate for the "GABA transporter" [21], the drug is believed to enter cells via some high affinity uptake system [17].

With recent evidence proposing the existence of multiple GABA uptake carriers [14], it is possible that VGB has a specific action at one or more of these newly discovered targets. The GABA uptake carrier "GAT-3" might represent the most likely site of VGB action in this respect. Beta-alanine, a specific substrate of GABA-T [22], has been shown to selectively block GABA transport via the "GAT-3" carrier [23]. It is possible that structural similarities facilitate the binding of GABA, β-alanine and VGB at the active sites of both GABA-T and the "GAT-3" transporter.

In cultures exposed to a combination of both TGB and VGB, the inhibition of GABA uptake was greater than that observed when either drug was administered alone. This effect did, however, appear to be infra-additive and would thus suggest a similar site of action for both drugs. If TGB and VGB were to act at independent uptake sites one would expect to observe a total inhibition of GABA uptake equal to the sum of inhibitions observed with both drugs alone. This evidence might support a contributory role for VGB on the TGB-mediated blockade of GABA uptake at the "GAT-3" transporter.

In conclusion, these results suggest that both TGB and VGB block GABA uptake into primary cultures of rat cortical astrocytes. Further studies to determine the basis of the U-shaped dose-response to TGB and its lack of effect prior to 4 hours are required. In terms of VGB, a more detailed evaluation of this novel mechanism is planned. Further investigations of its dose- and time-dependency and cell specificity, together with evaluation of the relationship between this and the intracellular effects of VGB are clearly required. Characterisation of the GABA uptake blockade observed with VGB may help to determine its relative importance as an additional mechanism of anticonvulsant action.

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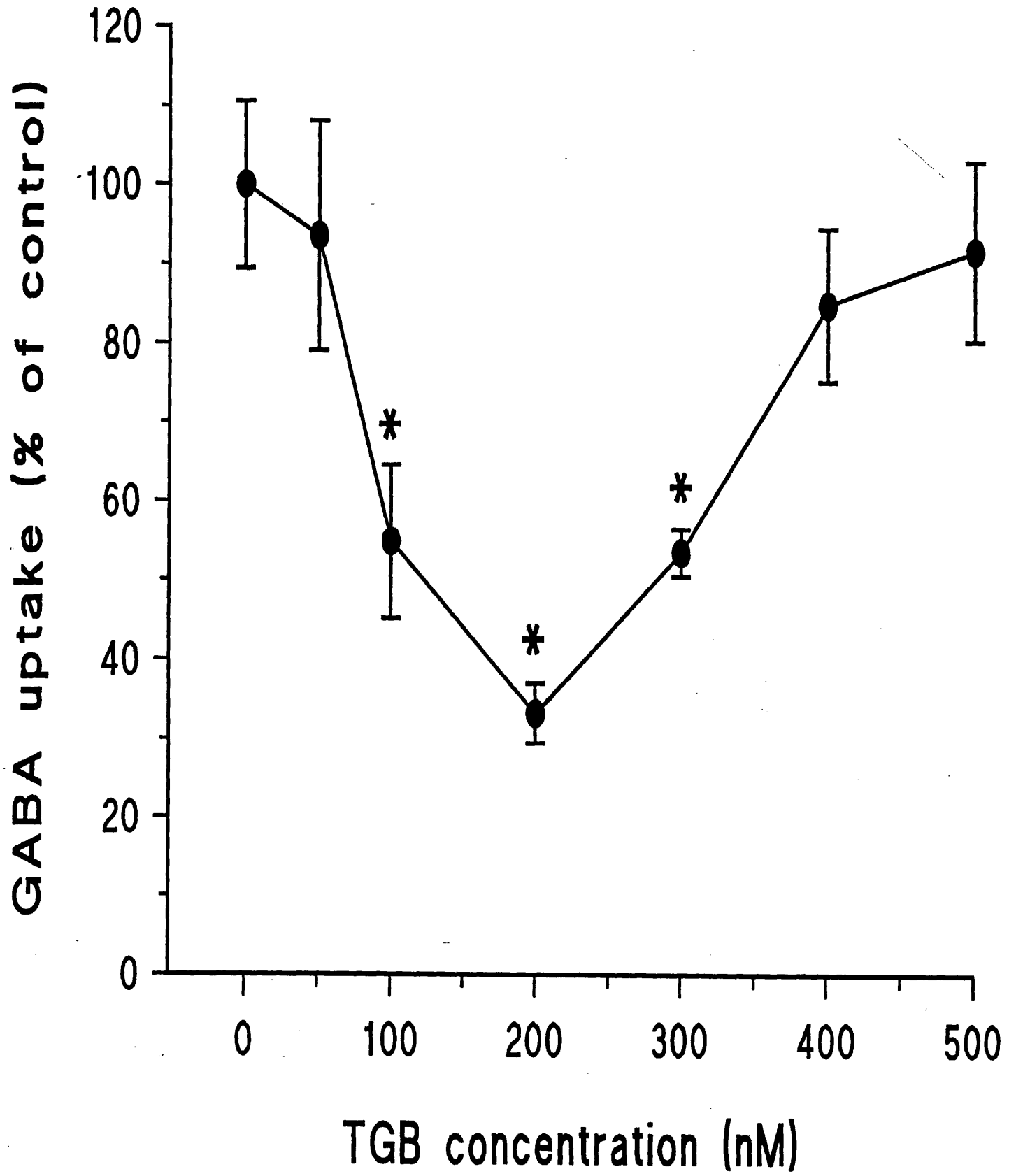
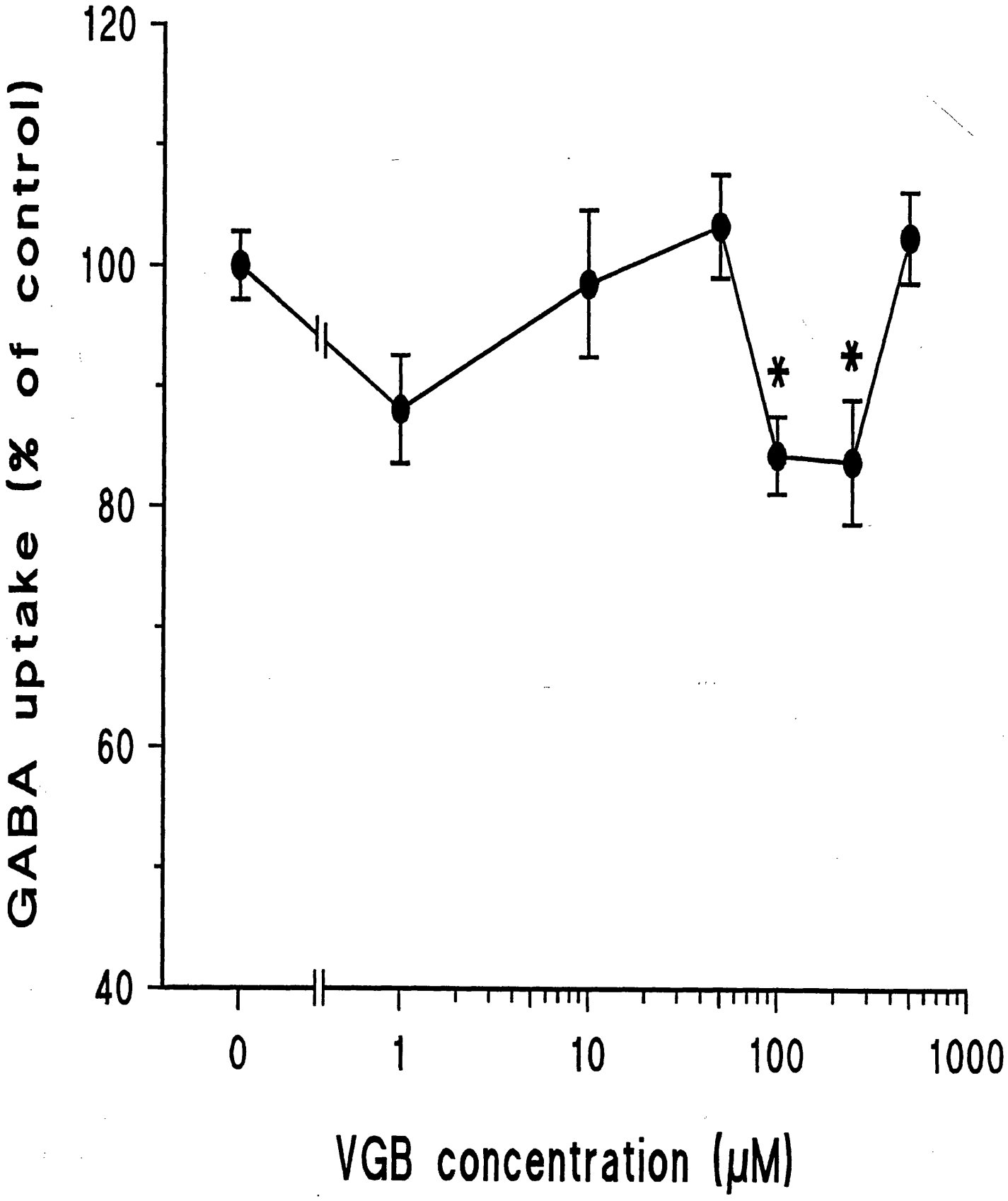
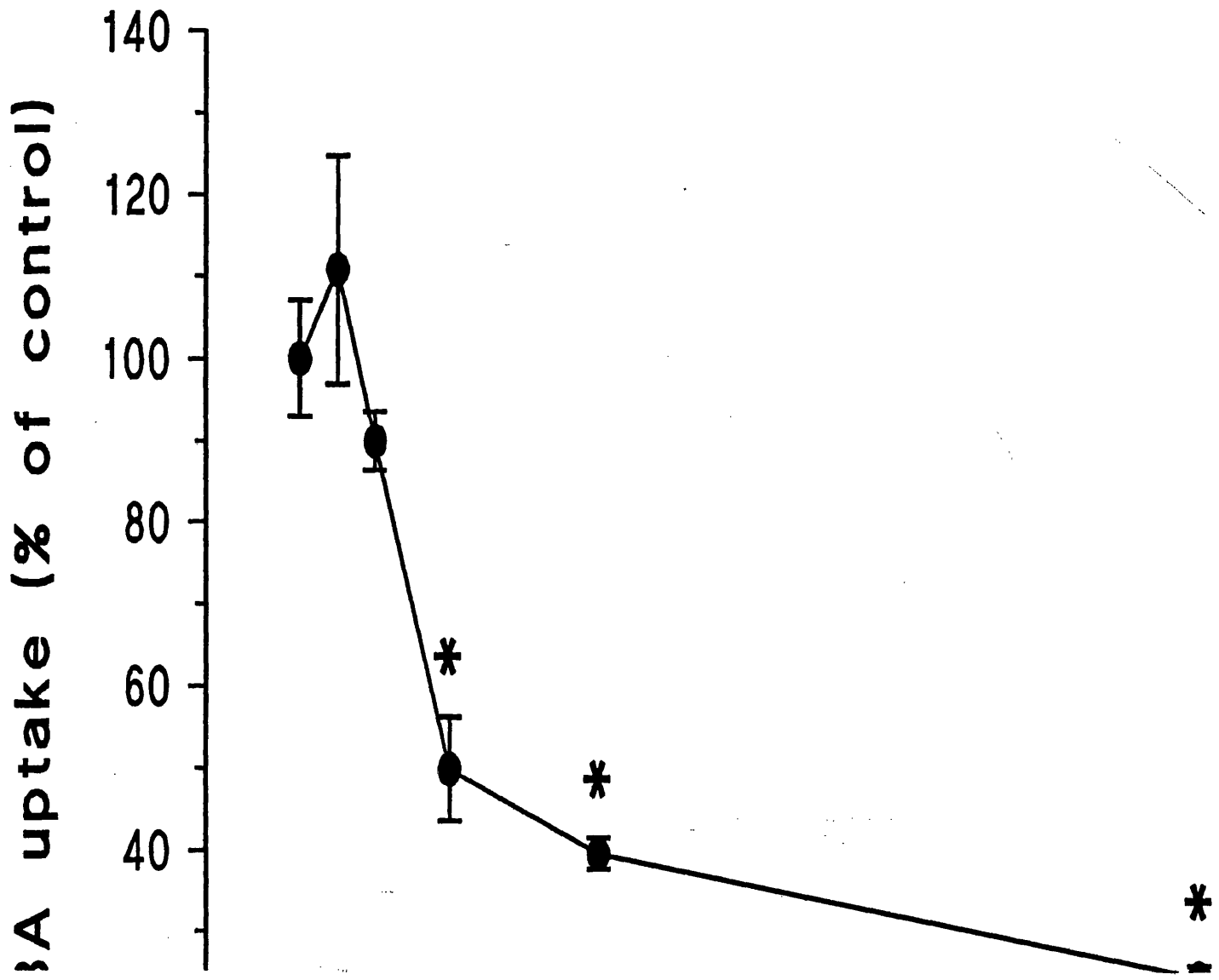
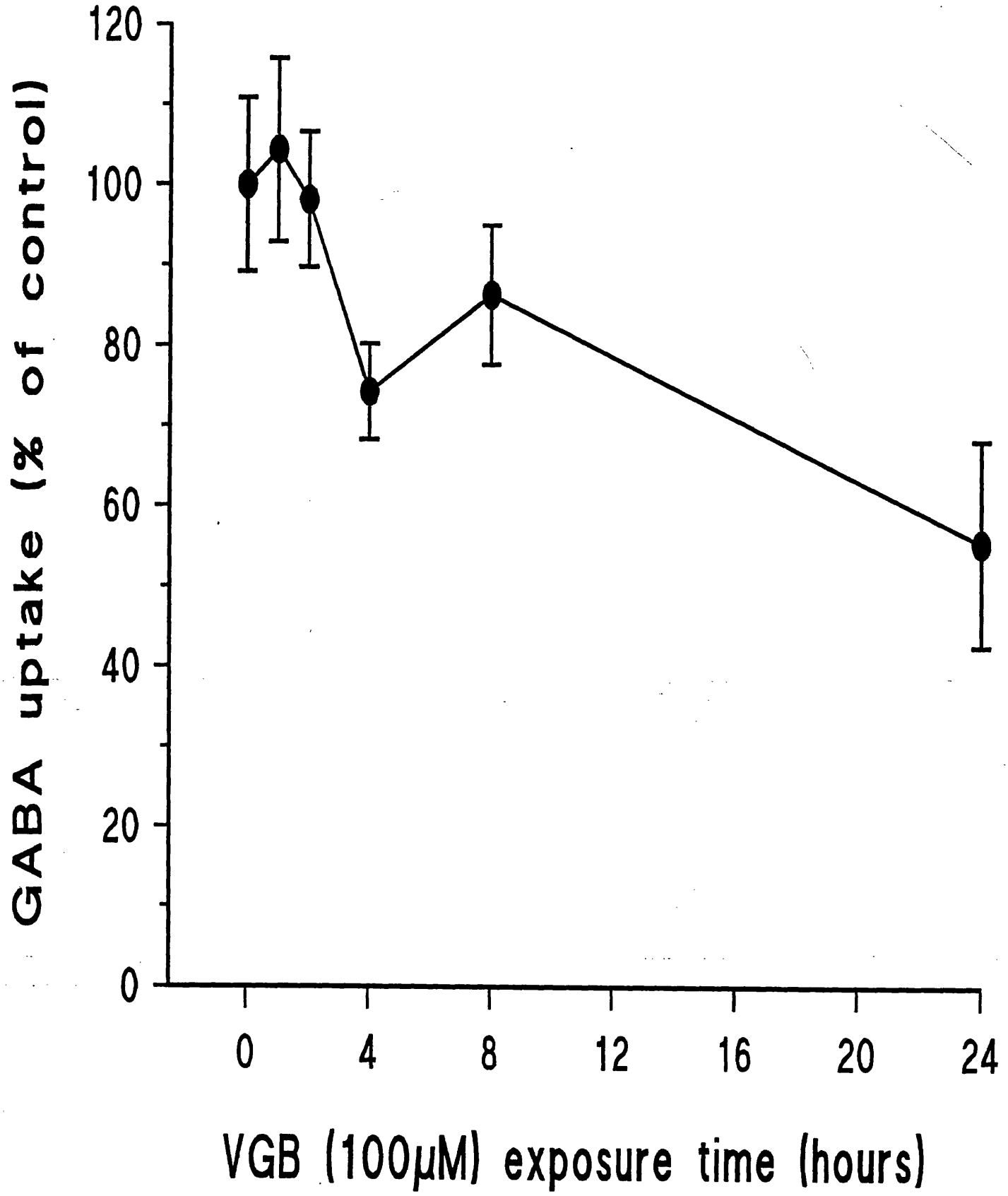
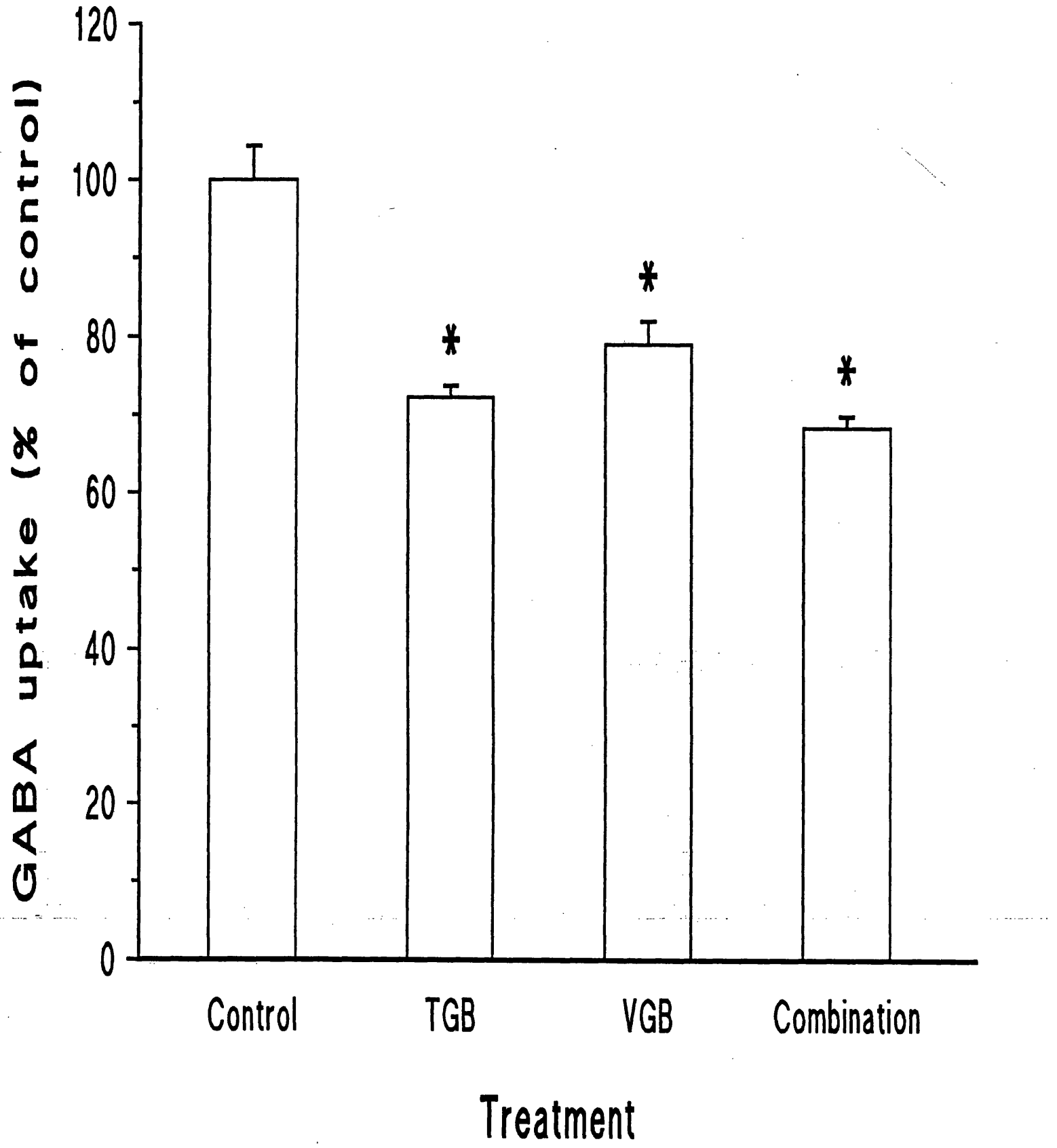

GABA uptake (% of control)
120
100
80
60
40
20
0
*
*
*
0
100
200
300
400
500
TGB concentration (nM)


GABA uptake (% of control)
120
100
80
60
40
0
1
10
100
1000
*
*
VGB concentration (μM)


140
120
100
80
60
40
uptake (% of control)
*
*
*


GABA uptake (% of control)
120
100
80
60
40
20
0
0
4
8
12
16
20
24
VGB (100μM) exposure time (hours)


GABA uptake (% of control)
120
100
80
60
40
20
0
*
*
*
Control
TGB
VGB
Combination
Treatment

LEGENDS TO FIGURES

Figure 1 :- Effects of tiagabine (TGB) concentration on the uptake of [^{14}C]-GABA into rat cortical astrocytes in primary culture following a four hour exposure. Results ($12 < n > 20$) are expressed as the mean percentage of the control values and error bars denote the standard error of the mean (SEM). Statistical significance ($*p<0.05$) was determined by one-way analysis of variance with Dunnett correction.

Figure 2 :- Effects of vigabatrin (VGB) concentration on the uptake of [^{14}C]-GABA into rat cortical astrocytes in primary culture following a four hour exposure. Results ($12 < n > 20$) are expressed as the mean percentage of the control values and error bars denote the standard error of the mean (SEM). Statistical significance ($*p<0.05$) was determined by one-way analysis of variance with Dunnett correction.

Figure 3 :- Effects of exposure time (hours) to tiagabine (TGB; 200 nM) on the uptake of [^{14}C]-GABA into rat cortical astrocytes in primary culture. Results ($12 < n > 20$) are expressed as the mean percentage of the control values and error bars denote the standard error of the mean (SEM). Statistical significance ($*p<0.05$) was determined by one-way analysis of variance with Dunnett correction.

Figure 4 :- Effects of exposure time (hours) to vigabatrin (VGB; 100 μM) on the uptake of [^{14}C]-GABA into rat cortical astrocytes in primary culture. Results ($12 < n > 20$) are expressed as the mean percentage of the control values and error bars denote the standard error of the mean (SEM). Statistical significance ($*p<0.05$) was determined by one-way analysis of variance with Dunnett correction.

Figure 5 :- Effects of tiagabine (TGB; 200 nM) and vigabatrin (VGB; 100 μM), alone and in combination, on the uptake of [^{14}C]-GABA into rat cortical astrocytes in primary culture following a four hour exposure. Results ($12 < n > 20$) are expressed as the mean percentage of the control values and error bars denote the standard error of the mean (SEM). Statistical significance (*$p<0.001$) was determined by two-sample t-test.

Mutual interaction between remacemide hydrochloride and carbamazepine: two drugs with active metabolites

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SUMMARY

A randomised, double-blind, placebo-controlled crossover study of add-on remacemide hydrochloride was carried out in 10 out of 14 recruited patients being treated with carbamazepine (CBZ) monotherapy. Forty eight hour concentration profiles of CBZ, its active epoxide metabolite (CBZ-E), remacemide, and its desglycinyl metabolite (ARL12495XX) were assayed following single and multiple dosing. Following 14 days' treatment with 300mg remacemide hydrochloride twice daily, the mean AUC of carbamazepine was increased by 22% ($p = 0.12$), Cmax by 27% ($p = 0.07$) and Cmin by 22% ($p = 0.29$). Trough concentrations of CBZ were statistically significantly higher ($p=0.0013$) during active treatment compared with placebo. Levels of CBZ-E were unaffected. No symptoms of carbamazepine toxicity were reported. There was no evidence of autoinduction of remacemide metabolism. However, in these CBZ-treated patients, exposure to remacemide and its active metabolite was 60% and 30% respectively of values observed in healthy volunteers treated previously with the same dose. Thus, remacemide hydrochloride inhibits CBZ metabolism, which itself induces that of remacemide hydrochloride and its active metabolite. This mutual interaction between remacemide hydrochloride and CBZ is predictable and modest and should not present a barrier to their clinical use in combination.

INTRODUCTION

Remacemide hydrochloride (2-amino-N-[1-methyl-1,2-diphenylethyl]-acetamide monohydrochloride), a novel anticonvulsant, is a non-competitive antagonist at N-methyl-D-aspartate (NMDA) receptors (1). Metabolism in man involves production of an active desglycinyl metabolite ARL12495XX (2), which has a longer elimination half-life (11-19 hours versus 3-4 hours) than the parent compound (3), and is a potent antagonist at the NMDA receptor (4). As well as being an anticonvulsant (5), remacemide hydrochloride could be effective in preventing cell damage (6) in the course of ischaemic injury (7) and in Parkinson's disease (8).

Carbamazepine (CBZ) is a first line anticonvulsant drug (9). Its use is complicated by variable autoinduction of metabolism (10) and a propensity for pharmacokinetic drug interactions (11). Hepatic metabolism of CBZ in man produces an active 10,11 epoxide (CBZ-E) metabolite, which is thought to be responsible for some of its neurotoxic side-effects (12). Metabolism of this compound is also affected by other antiepileptic drugs (13). The predilection of the established antiepileptic drugs to interact with other compounds and the need for novel agents to be used as adjuvant therapy require early assessment of potential pharmacokinetic interactions, which can obscure interpretation of trial results and influence dose selection (14). This pharmacokinetic interaction study with remacemide hydrochloride was carried out in patients on CBZ monotherapy, using a randomised, double-blind placebo-controlled crossover design (15).

METHODS

Patients

A total of 14 patients were recruited (Table 1), each on a regimen of CBZ monotherapy which had been stable for at least three months. All patients had at least two plasma measurements of CBZ within the target range (20-50umol/L) during that period. Twelve of the patients had partial seizures

with or without secondary generalisation, while 2 were thought to have idiopathic tonic-clonic seizures. The study was approved by both the West Ethical Committee in Glasgow and the Research Ethics Committee in Cardiff. Written informed consent was obtained from each participant

All patients were free from hepatic, renal, or haematological disease. They were asked to refrain from alcohol and caffeine throughout the study. Some patients were on stable doses of other medication, none of which was known to interact with CBZ or remacemide hydrochloride. Patients were required to keep a diary card detailing seizure type and frequency and the duration and nature of any adverse events experienced. Compliance was checked by questioning the patients and carrying out a tablet count.

Protocol

The study had a double-blind, random order, placebo-controlled, crossover design, preceded by an open, single-dose treatment phase. Patients continued to take CBZ in their usual dose throughout. One week following a screening visit, each patient received a single dose of 300mg remacemide hydrochloride. Plasma levels of CBZ, CBZ-E, remacemide, and ARL12495XX, were measured 0, 0.5, 1, 1.5, 2, 4,6, 8,10, 12, 24, and 48 hours after dosing. One week later, patients entered the first arm of chronic treatment, receiving either remacemide hydrochloride (100mg twice daily on day 1, 200mg twice daily on day 2, and 300mg twice daily thereafter) or matched placebo. The total treatment period was 14 days, after which the final dose of remacemide hydrochloride was given on the morning of the 15th day to allow measurement of washout concentrations at the same times after dosing as before. Seven days later, the second treatment phase was commenced and the whole procedure was repeated. Morning pre-dose (trough) samples were taken on the 5th, 12th and 15th day after initiation of treatment. All blood samples were taken into heparinised tubes from a cannulated forearm vein, which was kept patent between aspirations with normal saline. On each

occasion, the first 1ml withdrawn was discarded, and the subsequent 15ml were chilled until centrifugation. All samples were spun at 3,000 rpm for 10 minutes, and the separated plasma frozen at -4°C for batch analysis.

Assays

Remacemide and its desglycinyl metabolite (ARL12495XX) were quantified by high performance liquid chromatography (HPLC). This was a modification of a previously reported method (16), adjusted to allow automated sample preparation and improve selectivity. The method involved solid phase extraction followed by separation on a reverse phase HPLC system utilising a octadecyl (C-18) HPLC column, an acetonitrile based eluent, and ultraviolet (uv) detection at 210 nm. Limits of quantification for the two analytes were 10 ng/ml. Only samples from the active leg of the double-blind phase were analysed for remacemide and ARL12495XX. CBZ and CBZ-E were measured by liquid-liquid extraction of plasma followed by reverse phase HPLC utilising a C-8 column, a methanol-based mobile phase, and uv detection at 210nm.

Pharmacokinetics

The following non-compartmental pharmacokinetic parameters were computed for CBZ and CBZ-E for all three phases of serial blood sampling:

1) area under the concentration-time curve (AUC) over a dosing interval (AUC_{0-T}) calculated using the linear trapezoidal method
2) peak concentration (Cmax) over the dosing interval
3) trough/pre-dose concentration (Cmin) over the dosing interval
4) time to maximum concentrations (Tmax)
5) Cmin 5, 12, and 15 days after initiation of multiple dosing with remacemide hydrochloride or placebo

For remacemide and ARL12495XX, the parameters calculated following single dose and multiple dosing were:

1) Cmax

2) Tmax

3) AUC, either extrapolated to infinite time (AUC_∞), which was calculated from AUC = AUCt + Ct/kel where AUCt = area under the curve up to the last point at which the concentration could be quantified, and kel = the terminal phase plasma elimination rate constant, or over a 12 hour dosing interval ($AUC_{0\text{-}12h}$)

4) Elimination half-life, ($t_{½}$) after the single dose and during washout of the multiple dose remacemide hydrochloride treatment phase. This was calculated from $t_{½}$ = 0.693/kel.

5) Cmin 5, 12 and 15 days into remacemide hydrochloride treatment.

Statistics

Statistical comparisons of the pharmacokinetic parameters obtained for CBZ and CBZ-E at the end of the two multiple dose phases of the study were compared using an analysis of variance (ANOVA) with treatment, period, sequence and patient as factors. Logarithmically transformed data were used for analysis of the AUC_T, Cmax and Cmin comparisons. Untransformed data were used for T max. Analysis of the trough concentrations used ANOVA for the 3 concentrations per patient (5, 12, and 15 days after initiation of multiple dosing) with factors of treatment, period, sequence, day number and patient. Single and multiple dose phases were compared using a non-parametric procedure, the Wilcoxon matched pairs signed rank test. A probability less than 5% indicated statistical significance.

RESULTS

Patients

Of the 14 patients recruited (Table 1), 10 completed the study as per protocol. One patient (018) withdrew a few hours following administration of the single remacemide hydrochloride dose because he disliked intravenous cannulation. Another (021) pulled out 5 days after single dose administration because of an intercurrent viral illness. One patient (024) was withdrawn because of suspected poor compliance, while the fourth (016) had the dose of remacemide hydrochloride halved following the onset of adverse events suspected to be due to the study drug. This patient's data were included in the summary of adverse events, but not in the pharmacokinetic analysis.

CBZ pharmacokinetics

There were no statistically significant changes in mean CBZ pharmacokinetic parameters following a single dose of remacemide hydrochloride (Figure 1 and Table 2). Following 14 days' treatment, the mean $AUC_{0\text{-}12h}$ of CBZ was increased by 22% ($p = 0.12$), the mean Cmax by 27% ($p = 0.07$), and the mean Cmin by 22% ($p = 0.29$). Tmax was unchanged following both single and multi-dose remacemide hydrochloride. Comparison of mean trough CBZ levels 5, 12, and 15 days after the start of active treatment (40.1, 34.4 and 40.4 umol/L respectively) with those on placebo (32.7, 30.5 and 34.5 umol/L respectively) showed a statistically significant increase ($p=0.0013$). Four patients had at least one of the pharmacokinetic parameters of CBZ increased by more than 30% during the remacemide hydrochloride treatment phase. None of these patients, however, reported any symptoms suggestive of CBZ toxicity.

CBZ-E pharmacokinetics

The mean AUC, Cmax, and Cmin for CBZ-E (Table 3) were not significantly altered by concomitant remacemide hydrochloride following single or multiple dosing (Figure 2). After 14 days' treatment,

two patients had an increase in AUC or Cmax of more than 30%, one being a rise in AUC of 177% and in Cmax of 153%. These patients, however, remained symptom-free. There was no significant difference in Tmax following acute or chronic remacemide hydrochloride dosing. Comparison of mean trough CBZ-E levels 5, 12, and 15 days after the start of active treatment (5.1, 4.4 and 5.0 umol/L respectively) with those during placebo treatment (4.9, 4.1, and 5.00 umol/L respectively) showed no significant differences ($p = 0.62$).

Remacemide and ARL12495XX pharmacokinetics

Mean pharmacokinetic parameters for remacemide and ARL12495XX following single and multiple dosing are shown in Table 4. Mean plasma concentrations following single and multiple dosing are illustrated for remacemide in Figure 3 and for ARL12495XX in Figure 4. As anticipated from a drug with a considerably shorter half-life than dosing interval, there was little carry-over of remacemide from dose to dose, and the steady-state profiles attained consisted of levels only slightly higher than those following the single dose (Figure 3 and Table 4). For ARL12495XX, however, consistent with its longer terminal half life, there was a greater carry-over during multiple dosing. At steady-state, peak (Cmax) and trough (Cmin) oscillations were much smaller than for remacemide, and the maximum concentrations attained were approximately twice those following the single dose (Figure 4 and Table 4). With both remacemide and ARL12495XX, there was good predictability of multiple dose profiles compared with single dose profiles based on linear superposition of the concentration data and comparisons of AUCs following single and multiple dosing. This suggests that there was no autoinduction of remacemide or ARL12495XX metabolism.

Adverse events

No major adverse events were reported, and no patients were withdrawn from the study due to adverse events, although one patient (016) had his dose of remacemide hydrochloride halved 4 days

into the multiple-dose phase because of dizziness. Overall, more events were reported while patients were on placebo (36 adverse events) than following remacemide hydrochloride treatment (26 events reported). Similar numbers of patients reported adverse events following multiple dosing with active treatment (10 patients) as were reported with placebo (9 patients). There were similar numbers of central nervous system events (4 on remacemide hydrochloride versus 3 on placebo), and gastrointestinal symptoms reported were equal with both treatments (2 each).

DISCUSSION

CBZ is a well-known inducer of hepatic cytochrome P450 mono-oxygenase enzymes (11). This results in marked intra- and inter-individual variations in serum concentrations of other antiepileptic drugs during polypharmacy, an unpredictability which is exacerbated by variable autoinduction of metabolism (10). In addition, many other drugs have been shown to interact pharmacokinetically with CBZ (14). Its clearance in man is almost exclusively by hepatic metabolic transformation. A major pathway involves oxidation of CBZ to 10,11 epoxide (CBZ-E), which is itself biotransformed by the enzyme epoxide hydrolase to the inert dihydrodiol (17). These two processes provide a target for interactions between CBZ and other antiepileptic drugs (13,18-19). Changes in the concentration of CBZ-E may be important because this compound contributes to the efficacy and adverse events associated with CBZ treatment (12). In one study the majority of patients on co-medication with remacemide hydrochloride and CBZ had dose related increases in trough concentrations of CBZ, necessitating a reduction in the CBZ dose in a few patients (20).

Like CBZ, remacemide is also eliminated almost exclusively by metabolic transformation. Apart from the active metabolite, ARL12495XX, which is formed by ubiquitous aminopeptidase enzymes, there are a number of oxidative biotransformation products. In addition, remacemide hydrochloride undergoes direct glucuronidation to form a carbamoyl glucuronide metabolite, an important pathway

in man (20). This interaction study used a placebo-controlled design in order to investigate the potential interaction between remacemide hydrochloride and CBZ. Following multiple dosing with remacemide hydrochloride, there was an overall small inhibition of CBZ metabolism during active treatment compared with placebo. A minority of patients demonstrated a rise in CBZ trough levels >30%. There were no significant changes overall in CBZ-E concentrations during treatment with remacemide hydrochloride, although there were marked differences in individual response with one patient exhibiting an increase in CBZ-E level of more than 100%. No patients had any clinical sequelae.

These findings are consistent with in-vitro experiments using 6-B-hydroxylation of testosterone as a marker of CYP3A4 activity (Riley - manuscript in preparation), which showed that remacemide is an inhibitor of cytochromal activity associated with this isoform. Since the concentrations of remacemide required in the in-vitro mixture to achieve inhibition of CYP3A4 are in excess of those reached in vivo, it is fair to assume that any increase in CBZ concentrations will be modest. Remacemide has not been shown to affect epoxide hydrolase in vitro, in keeping with the findings in the study.

This study also offered the opportunity to explore the pharmacokinetics of remacemide and ARL12495XX in enzyme-induced patients. For both compounds, the multiple dose profile was consistent with that predicted from the single dose, indicative of linear disposition and the absence of autoinduction of metabolism. The terminal half life of remacemide was similar to that found in previous clinical studies in human volunteers (Figure 5), whereas that of ARL12495XX in enzyme-induced patients was shorter than in untreated healthy volunteers (20). Exposure to remacemide based on AUC values was around 60% of that reported previously in non-induced subjects taking the same dose of the drug, while that of ARL12495XX was about 30% (Figure 5).

The most common adverse events reported with remacemide hydrochloride are dizziness and mild to moderate gastrointestinal upset (20). There were few adverse events reported in this study. In particular, there was none resulting in withdrawal from the study, although one patient developed dizziness, necessitating a decrease in remacemide hydrochloride dose. A greater number of adverse events were reported during the placebo phase. In healthy volunteers, the AUC following similar dosing with remacemide hydrochloride (600mg/day) was between two and three times higher than that observed in patients in this study. Consequently, 600mg remacemide hydrochloride per day is unlikely to be the maximum tolerated dose in patients receiving treatment with CBZ.

Although vigilance should be exercised in adding remacemide hydrochloride to antiepileptic drug regimes containing CBZ, it is unlikely that a reduction in CBZ dosage will be required in most patients. Since the presence of CBZ will result in lower bioavailability of remacemide and ARL12495XX, patients pre-treated with an enzyme-inducer such as CBZ will require higher doses of remacemide hydrochloride than non-induced patients. In addition, the remacemide concentration can be expected to rise when carbamazepine is withdrawn. The mutual interaction between CBZ and remacemide hydrochloride is predictable and modest, and should not present a barrier to their wide-spread clinical use in combination. As remacemide and ARL12495XX exhibit predictable and linear kinetics in CBZ patients, with no evidence of autoinduction, there should be little need for routine therapeutic monitoring of either drug in this clinical setting.

Acknowledgement

Our thanks go to Mrs Moya Dewar for expert secretarial assistance.

Enclosure (vi)

Table 1

Demographic characteristics of study population

Patient	Sex	Age (years)	Seizure type	CBZ dose (mg/day)	Treatment schedule
001	M	36	CPS/2GTCS	600	tid
002	M	47	SP/2GTCS	800	tid
003	M	48	CPS/2GTCS	800	bd
016	F	65	CPS/2GTCS	800	bd
017	F	43	CPS/2GTCS	800	bd
018	M	46	2GTCS	400	bd
019	M	56	SP/CPS	600	tid
020	M	39	CPS/2GTCS	600	od
021	F	57	2GTCS	400	bd
022	F	46	SP/CPS/2GTCS	1200	bd
023	M	57	SP/CPS/2GTCS	800	bd
024	F	51	1GTCS	800	bd
025	F	40	CPS/2GTCS	1200	od
027	F	40	1GTCS	1600	bd

SP = simple partial seizures

CPS = complex partial seizures

1GTCS = idiopathic tonic-clonic seizures

2GTCS = localisation-related tonic-clonic seizures

Table 2

Mean carbamazepine pharmacokinetic parameters (SD) after single and multiple doses of remacemide hydrochloride and placebo in 10 epileptic patients

	AUC ($umol.hr.l^{-1}$)	Cmax ($umol.l^{-1}$)	Tmax (hours)	Cmin ($umol.l^{-1}$)
Placebo	367.8 (135.2)	40.5 (8.7)	4.6 (4.8)	34.7 (8.2)
Single dose	392.3 (161.1)	43.3 (10.1)	7.1 (4.2)	34.0 (7.2)
Multiple doses	425.4 (156.9)	50.6 (12.2)	5.1 (3.5)	40.8 (11.6)

Table 3

Mean carbamazepine 10,11 epoxide pharmacokinetic parameters (SD) after single and multiple dose of remacemide hydrochloride and placebo in 10 epileptic patients

	AUC ($umol.hr.l^{-1}$)	Cmax ($umol.l^{-1}$)	Tmax (hours)	Cmin ($umol.l^{-1}$)
Placebo	53.8 (32.7)	5.6 (2.6)	6.2 (5.1)	5.0 (2.4)
Single dose	46.7 (26.9)	5.0 (2.2)	4.6 (4.6)	4.5 (2.2)
Multiple doses	48.4 (27.5)	5.9 (2.4)	5.2 (4.6)	4.9 (2.2)

Table 4

Mean pharmacokinetic parameters (SD) of remacemide and ARL12495XX in 10 carbamazepine treated patients following acute and chronic dosing.

		Cmax ($ng.ml^{-1}$)	Cmin ($ng.ml^{-1}$)	Tmax (hours)	AUC* ($ng.hr.ml^{-1}$)	t½ (hours)
Single dose						
	Remacemide	783 (229)	NA	1.5 (1.0)	2266 (1344)	3.60 (1.31)
	ARL 12495XX	30.2 (7.6)	NA	2.0 (0.7)	395 (125)	10.44 (0.65)
Multiple doses						
	Remacemide	1006 (411)	60.9 (74.8)	1.1 (0.4)	2644 (1376)	3.54 (1.47)
	ARL 12495XX	64.8 (23.2)	25.2 (7.9)	1.6 (0.9)	427 (108)	11.25 (4.11)

*AUC_{∞} for single dose profiles, $AUC_{0\text{-}12h}$ for multiple dose profiles NA = not applicable

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Legends to figures

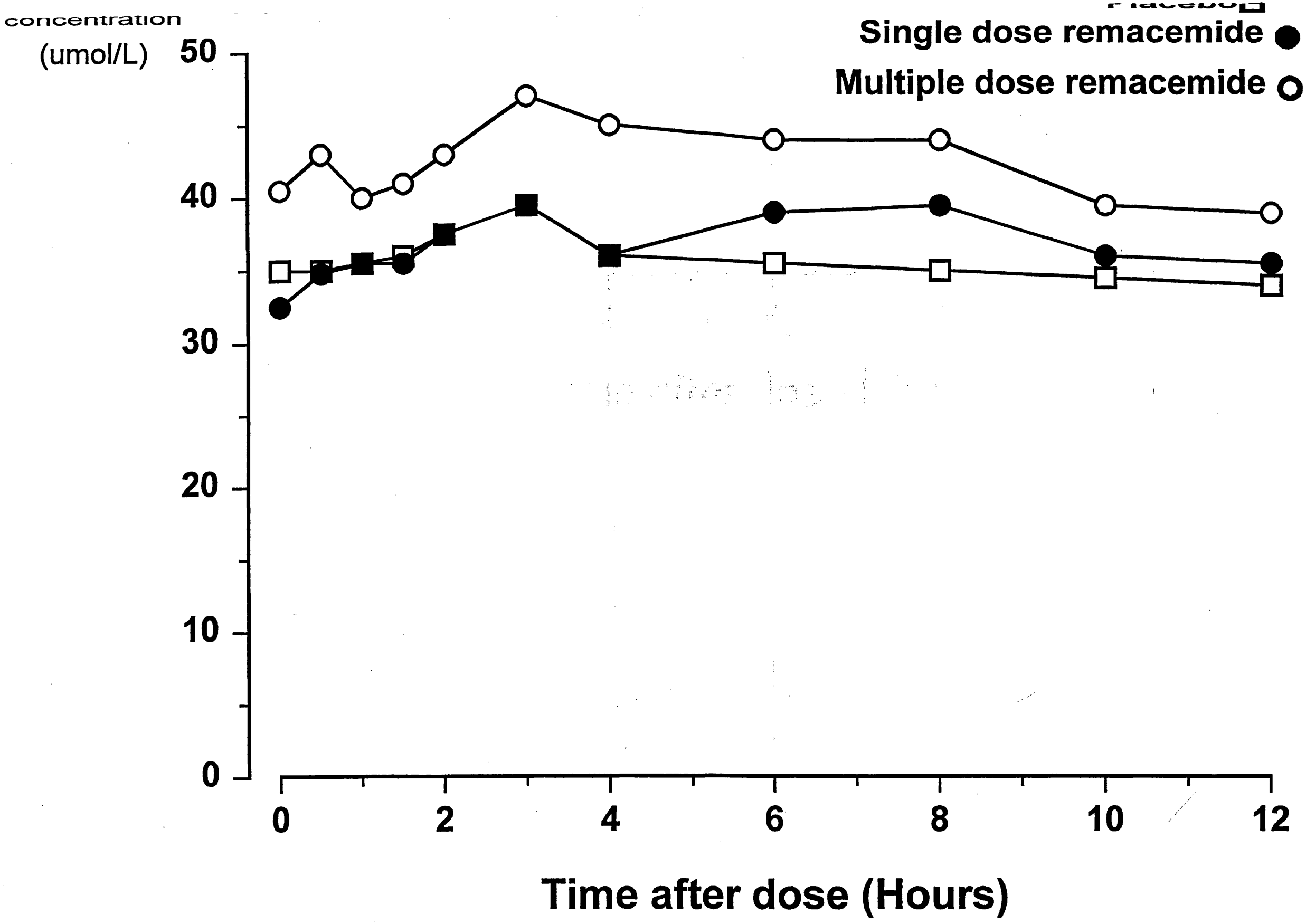
Figure 1 Mean plasma concentrations of carbamazepine in 10 patients with epilepsy following single (300mg) or multiple doses (300mg twice daily for 14 days) of remacemide hydrochloride and multiple dose placebo

Figure 2 Mean plasma concentrations of carbamazepine 10,11-epoxide in 10 patients with epilepsy following single (300mg) and multiple doses (300mg twice daily for 14 days) of remacemide hydrochloride and multiple dose placebo

Figure 3 Mean (±SD) single dose and steady-state plasma concentrations of remacemide in 10 patients taking carbamazepine who received a single dose (300mg) or multiple (300mg twice daily for 14 days) doses of remacemide hydrochloride

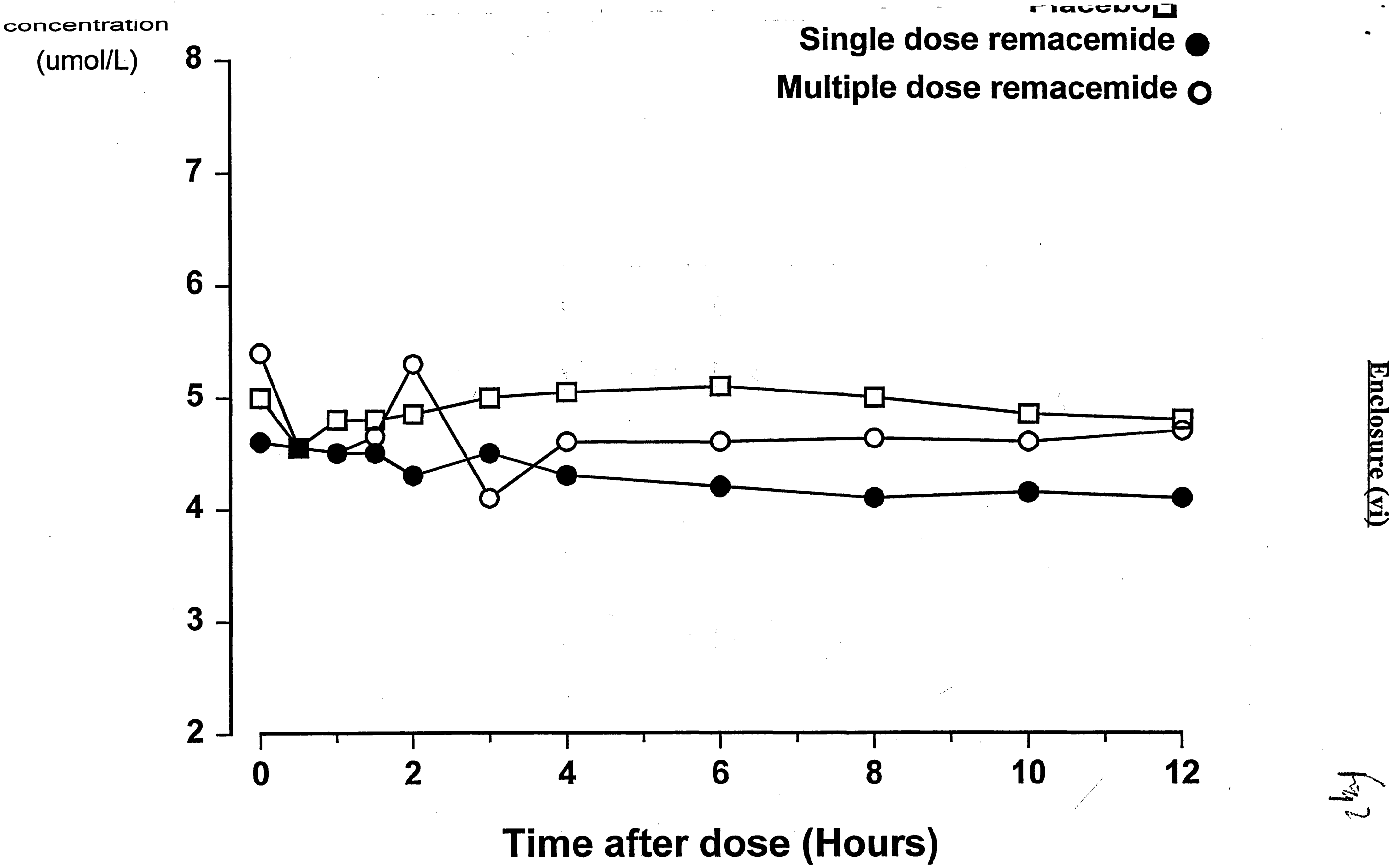
Figure 4 Mean (±SD) single dose and steady-state plasma concentrations of ARL12495XX in 10 patients taking carbamazepine who received a single dose (300mg) or multiple (300mg twice daily for 14 days) doses of remacemide hydrochloride

Figure 5 Mean pharmacokinetic parameters of remacemide and ARL12495XX in carbamazepine-treated patients versus healthy untreated volunteers (volunteer data taken from reference 20)



Enclosure (vi)

fig 1



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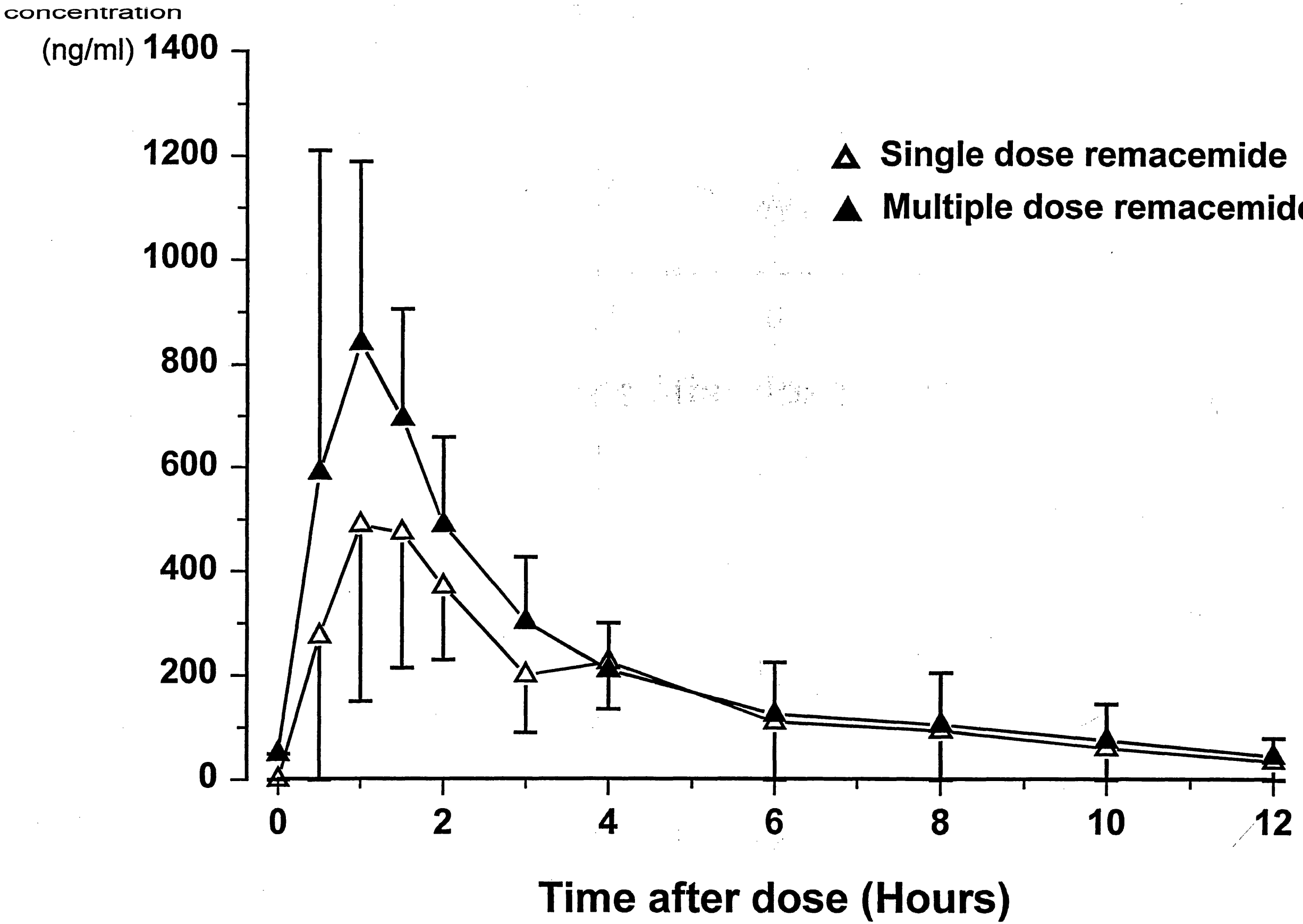

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Hyg 3

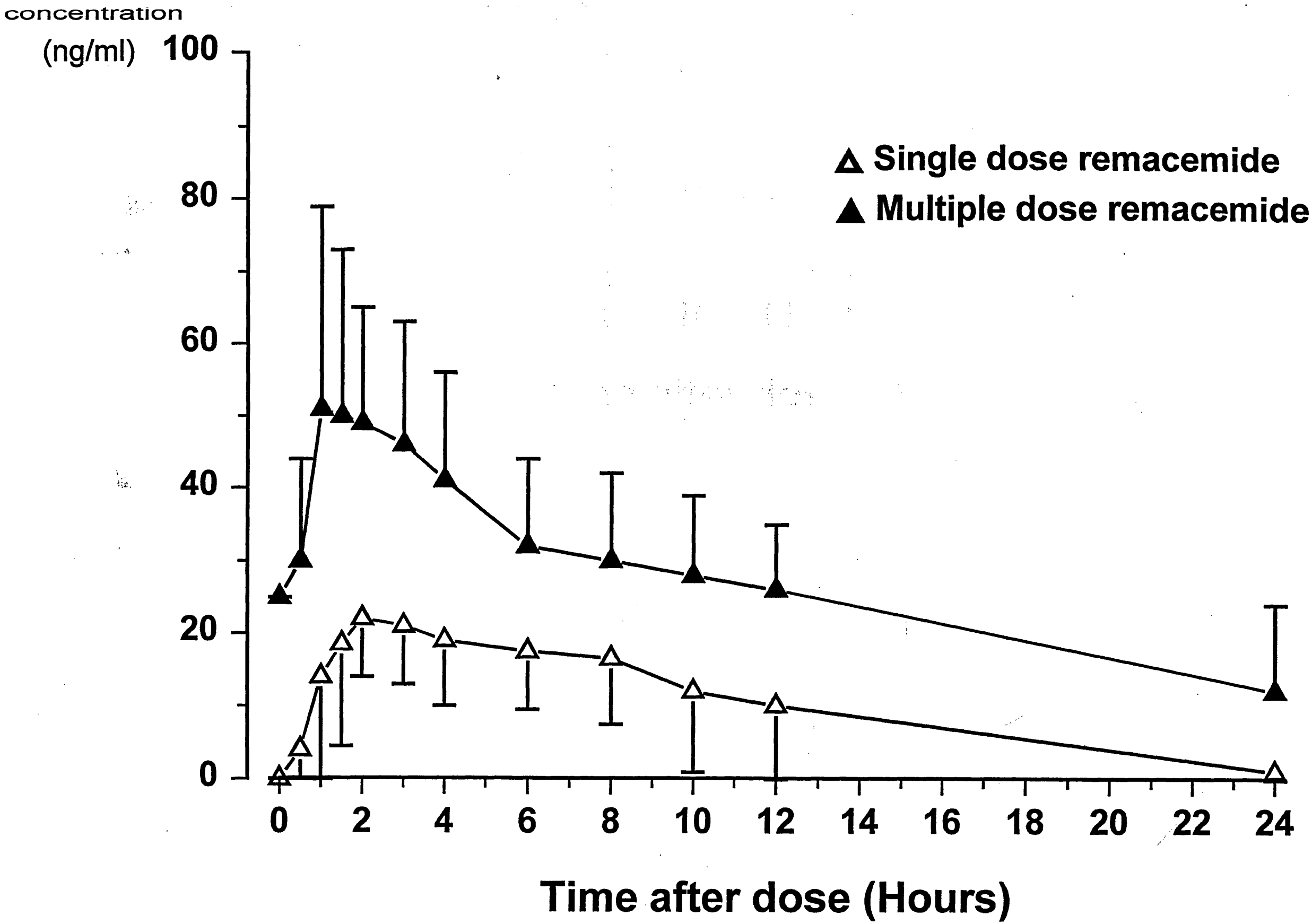

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Fig 5