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The pharmacokinetics and neuropharmacological action of the new antiepileptic drugs vigabatrin and levetiracetam

Thesis submitted for the degree of Doctor of Philosophy to the Faculty of Medicine of the University of London

By

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ABSTRACT

Epilepsy affects approximately 1% of the world population and is the most serious neurological conditions. In the UK, 30-35,000 new cases of epilepsy are diagnosed each year, resulting in a prevalence of 300,000 nationwide. There is significant need for new drug treatments. Yet we have a poor understanding of how many of these drugs mediate their antiepileptic effect, and how and where they are distributed within the brain. This thesis sought to investigate the pharmacokinetic and neuropharmacokinetic inter-relationship and the neuropharmacology of two new antiepileptic drugs, vigabatrin and levetiracetam; two drugs with distinct mechanisms of action.

Firstly, a freely moving and freely behaving rat model was used to determine the pharmacokinetics of vigabatrin and levetiracetam simulatneously in serum, cerebrospinal fluid (CSF) and brain frontal cortex and hippocampal extracellular fluid (ECF). Secondly, amino acid neurotransmitter concentrations were monitored in CSF (by direct CSF sampling) and brain ECF (by microdialysis) after vigabatrin and levetiracetam administration. Thirdly, the effects of vigabatrin and levetiracetam on paired-pulse inhibition recorded in the dentate gyrus evoked by perforant path stimulation were established.

Vigabatrin rapidly entered the blood (serum), CSF and ECF (frontal cortex and hippocampus) compartments with concentrations increasing linearly and dose-dependently. Time to maximum concentration (T_{max}) was achieved at a mean value of 0.4 ± 0.06 minutes in the blood compartment, 0.9 ± 0.1 minutes in the CSF compartment and 0.8 ± 0.1 minutes in both the frontal cortex and hippocampal ECF compartments. Although the CSF kinetics of vigabatrin paralleled that seen in serum, CSF vigabatrin concentrations represented only 2% of serum vigabatrin

concentrations and did not reflect free drug concentrations in serum. Vigabatrin was not protein bound in serum. Furthermore, the efflux of vigabatrin from the CSF compartment was significantly slower (mean terminal half life {$t_{1/2}$} values, 2.2-3.3 h) than that suggested by serum values (mean $t_{1/2}$ values, 1.1-1.4 h). Distribution in the brain ECF was brain region specific; vigabatrin concentrations achieved in the frontal cortex were 2-fold greater than concentrations achieved in the hippocampus. However, the efflux of vigabatrin from the two brain regions was essentially identical (mean $t_{1/2}$ values, 2.4-3.6 h) and indeed was similar to values seen in the CSF compartment. The findings are consistent with an active uptake and elimination of vigabatrin from the CSF and ECF.

Levetiracetam rapidly entered the blood (serum) and ECF (frontal cortex and hippocampus) compartments with concentrations increasing linearly and dose-dependently. Mean T_{max} values were 0.4 – 0.7 minutes in the blood compartment and 1.8 – 2.5 minutes in both the frontal cortex and hippocampal ECF compartments. Levetiracetam was not protein bound in serum. In contrast to vigabatrin, levetiracetam did not exhibit brain region specificity in that its neuropharmacokinetic profiles in frontal cortex and hippocampal ECF were essentially identical. However, the efflux of levetiracetam from the two brain regions was slower (mean $t_{1/2}$ values, 3.1 – 3.3 h) compared to that which occurred in the blood compartment (mean $t_{1/2}$ values, 2.2 h).

In the CSF compartment, vigabatrin administration was associated with changes in 5 of the 16 amino acid neurotransmitter concentrations measured over time. Thus whilst arginine and tyrosine concentrations decreased, homocarnosine, glycine and taurine concentrations increased. Although γ-aminobutyric acid (GABA) was not measured in CSF, the fact that homocarnosine (a GABA conjugate) concentrations increased is

consistent with a GABAergic action for vigabatrin. In the frontal cortex and hippocampal ECF compartments, vigabatrin administration was associated with significant changes in various amino acid concentrations but the changes did not parallel those seen in CSF. The most profound change was that with GABA. However, whilst ECF GABA concentrations increased 6-fold in the frontal cortex, concentrations in the hippocampus were unaffected. These GABA changes did not parallel the concentration versus time profile of ECF vigabatrin; nevertheless vigabatrin concentrations in the frontal cortex were 2-fold higher than those achieved in the hippocampus. These findings indicate that CSF amino acid measurements may be a poor reflection of ECF amino acid changes and that changes in ECF amino acids is regionally specific. That vigabatrin reduced paired pulse inhibition in the dentate gyrus evoked by perforant path stimulation at 20 ms interpulse interval but not at 50 ms and 100 ms intervals would suggest that vigabatrin by increasing extracellular GABA may either desensistise synaptic $GABA_A$ receptors or inhibit GABA release through an action on $GABA_B$ receptors.

In the brain ECF compartment, levetiracetam administration was associated with changes in only two (taurine and glutamate) of the 20 amino acid neurotransmitter concentrations measured over time. The significance of these changes in relation to the mechanism of action of levetiracetam is unknown. However, the fact that ECF GABA concentrations were unaffected by levetiracetam administration further indicates that levetiracetam has no effect on the GABAergic system. Indeed such a conclusion is reinforced by the observation that levetiracetam was without effect on paired-pulse inhibition.

In summary, both vigabatrin and levetiracetam have favourable kinetic features in that they exhibit linear dose-dependent pharmacokinetics and neuropharmacokinetics,

rapidly enter the central CSF and brain ECF compartments and that their efflux from these compartments are prolonged compared to that seen in serum. The brain distribution of vigabatrin is region specific (higher vigabatrin concentrations achieved in the frontal cortex versus the hippocampus), and this specificity is also reflected in relation to changes in ECF GABA concentrations; although the concentration versus time profiles for vigabatrin and GABA do not coincide and in fact are distinct. GABA is clearly a component of the mechanism of action of vigabatrin. In contrast levetiracetam neither affects ECF GABA concentrations nor appears to act via a GABAergic mechanism.

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LIST OF ABBREVIATIONS

AED	Antiepileptic drug
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
AUC	Area under of the concentration versus time curve
BBB	Blood-brain barrier
CI	Clerance
C_{max}	Maximum concentration
CNS	Central nervous system
CSF	Cerebrospinal fluid
DA	Dopamine
EAAs	Excitatory amino acids
ECF	Extracellular fluid
EEG	Electroencephalograph
EPSP	Excitatory postsynaptic potential
GABA	γ-Aminobutyric acid
GABA-T	GABA-transaminase
GAD	Glutamic acid decarboxylase
GDH	Glutamate dehydrogenase
GlyRs	Glycine receptors
HPLC	High performance liquid chromatography
IP	Intraperitoneal
IPI	Interpulse interval
IPSP	Inhibitory postsynaptic potential
IS	Internal standard
IV	Intravenous
KA	Kainic acid
MCPG	a-methyl-4-carboxyohenyl-glycien
MES	Maximal electroshock
mGluR	Metabotropic receptor

NMDA	*N*-methyl-D-aspartate
NMRS	Nnuclear magnetic resonance spectroscopic
PGP	P-glycoprotein
PHT	Phenytoin
PPI	Paired-pulse index
PS	Population spike
SE	Status epilepticus
SNR	Substanitial nigra reticulata
SSADH	Succinic semi-aldehyde dehydrogenase
$t_{1/2}$	Elimination half-life
T_{max}	Time to maximum concentration
TMS	Transcranial magnetic stimulation
Vd	Volume of distribution
VSSCs	Voltage-sensitive sodium channels

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1. **Tong X** & Patsalos PN (2001) A microdialysis study of the novel antiepileptic drug levetiracetam: extracellular pharmacokinetics and effect on taurine in rat brain. Br J Pharmacol,133: 867-874.
2. **X. Tong**, N. Ratnaraj and P.N.Patsalos (2007) The pharmacokinetics of vigabatrin in rat blood and cerebrospinal fluid. Seizure, 16: 43-49.

ABSTRACTS:

1. Patsalos PN, **Tong X** (2001) Levetiracetam extracellular pharmacokinetics and concureent amino acid neurotransmitter effects in rat frontal cortex and hippocampus using microdialysis. Epilepsia 42 (Suppl. 2):72.

2. **Tong X,** O'Connell MT, Ratnaraj N, Patsalos PN (2000) Vigabatrin and GABA concentration inter-relationship in rat extracellular fluid (ECF) frontal cortex and hippocampus. Epilepsis, 41 (Suppl Florence 2000):95.

3. **Tong X,** Walker MC, Patsalos PN (1999) Effects of vigabatrin on paired-pulse inhibition in vivo. Epilepsia, 40 (Suppl 9): 136.

4. **Tong X,** Ratnaraj N, Patsalos PN (1999) Extracellular fluid (ECF) kinetics of vigabatrin (VGB) in rat frontal cortex and hippocampus. Epilepsia, 40 (Suppl Florida): 28.

5. P.N.Patsalos, **X. Tong** and N. Ratnaraj (1999) Intracerebral microdialysis shows that vigabatrin does not exhibit ubiquitous brain extracellular distribution. Seizure, 8 (abstracts): 32.

6. **Tong X,** Ratnaraj N and Patsalos PN (1998) Vigabatrin affect on amino acid concentration in rat cerebrospinal fluid (CSF). Epilepsia, 39 (Suppl 6): 45.

7. **Tong X,** Ratnaraj N, Pataslos PN (1998) Serum pharmacokinetics and cerebrospinal fluid neuropharmacokinetics of vigabatrin in the rat. Epilepsia, 39 (Suppl 6): 43.

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DECLARATION:

All of the work described in this thesis was undertaken by the author, with the exception of the measurement of the amino acid concentrations by HPLC after vigabatrin and levetiracetam, which was undertaken by Dr M O'Connell.

Chapter 1

Introduction

The term "Epilepsy" is derived from the Greek word epilepsia and is the tendency to experience seizures of major or minor intensity and of varying characteristics. Epilepsy affects approximately 1% of the world population about 50 million people worldwide and is one of the most common serious neurological conditions. The incidence of epilepsy is 40-80 per 100,000 people per year and seizures are for the most part self-terminating (Hopkins, 1987). The life-time chance of developing epilepsy is 3-5% (Sander and Shorvon, 1987). However, on occasion, seizures can continue unabated and they are then considered as a separate entity, status epilepticus (SE). SE is one of the most common life-threatening neurological disorders.

The treatment goal for patients with epilepsy is to make them seizure free. The established antiepileptic drugs (AEDs) carbamazepine, ethosuximide, phenobarbitone, phenytoin and valproate, can render approximately 80% of newly diagnosed patients seizure-free; the remaining 20% of patients with refractory epilepsy often require polytherapy regimens involving two or more drugs. Seizure control is, however, far from optimal in those patients who receive multiple AEDs, and they are invariably faced with adverse effects that may be intolerable. Most patients with chronic intractable epilepsy therefore rely on the development of new AEDs as their only hope for seizure control.

Several developments have underpinned the acceleration in the evolution of new AEDs. Testing of compounds in animal models has enabled a greater understanding of the molecular basis of seizures, and of clinical pharmacokinetics. However, most of the new AEDs have been identified and developed consequent to

efficacy screening in animal models and without regards to any detailed knowledge of how they work (Loscher & Schmidt, 1993).

Understanding the principle of epilepsy and knowing the exact mechanism of action of a drug can result in a more rational approach to epilepsy management and seizure control. Therefore this thesis focuses on the pharmacokinetics, neuropharmacokinetics and the mechanism of action of two new AEDs, vigabatrin and levetiracetam, in animal models.

1.1 THE CLASSIFICATION AND DEFINITION OF EPILEPSIES

The most widely used classification of the epilepsies is the proposal from the Commission on Classification and Terminology of the International League Against Epilepsy (ILAE, 1981). Table 1 summarises the international classification of epileptic seizures. This classification has two major divisions, which are partial and generalised.

1.1.1 Partial seizures

Partial seizures originate in a focal region of the cortex and can be subdivided into those that do not impair consciousness, sample partial and those that do impair consciousness, complex partial. Partial seizure can spread rapidly to other cortical areas through neuronal networks, resulting in secondary generalized tonic-clonic seizures.

Table 1.1: International classification of epileptic seizure

Partial seizures (beginning locally)	**Generalized seizure (convulsive or non-convulsive)**
Simple partial (without impaired consciousness)	Absence - typical
- with motor symptoms	- atypical
- with somatosensory or special sensory symptoms	Myoclonic
- with autonomic symptoms	Clonic
- with psychic symptoms	Tonic
Complex partial (with impaired consciousness)	Tonic-clonic
- simple partial onset followed by impaired consciousness	Atonic
- impaired consciousness at onset	Unclassified
Partial, evolving into secondary Generalized seizures	

* Adapted from Commission Classification and Teminology of the international League Against Epilepsy. 1981

(Brodie et al, 2005)

1.1.1.1 Simple partial seizure

Simple partial seizures begin locally in the cortex and are often preceded by an aura which reflects the functional role of that part of the cortex in which the seizure begins. The symptoms and signs of simple partial seizures depend on the site of origin of the abnormal electrical discharge. Those arising from the motor cortex cause rhythmic movements of the contralateral face, arm or leg. Seizures arising

from sensory regions or areas responsible for emotions and memory may produce olfactory, visual or auditory hallucinations, fear, panic or euphoria (Brodie et al, 2005).

1.1.1.2 Complex partial seizures

Complex partial seizures are differentiated from simple partial seizures by varying degrees of impairment of consciousness and are frequently succeeded by post-ictal confusion. Complex partial seizures typically last less than 3 minutes. During that time, patients may appear awake, but lose contact with their environment and do not respond normally to instructions or questions. Patients usually stare and either remain motionless or engage in repetitive semi-purposeful behaviour called automatisms (Brodie et al, 2005).

1.1.2 Generalised Seizures

1.1.2.1 Tonic-clonic seizure

Tonic-clonic seizures are the most common form of generalised seizure. The patient initially has a tonic phase, associated with respiratory arrest; reflex emptying of the bladder and bowel may occur. Next the patient enters a clonic phase of rhythmic generalised jerking lasting for a variable length of time. Deep coma follows with an ascending conscious level which often includes a post-ictal phase of confusion and automatic behaviour. It takes 15-60 minute to become fully conscious. Afterwards people often complain of a generalised headache, a feeling of lethargy and a desire to sleep.

1.1.2.2 Myoclonic seizures

Myoclonic seizures occur in a number of differing epileptic syndromes. These seizures may be associated with both typical and atypical absences but more commonly occur without impairment of consciousness. The arms tend to be most frequently involved in a sudden flexion movement (Chadwick, 2003).

1.1.2.3 Absence seizures

Typical absence seizures

Typical absences last 5-10 seconds and commonly occur in clusters. They manifest as sudden onset of motor arrest, staring and impaired consciousness. This seizure type is associated with regular 3Hz spike-wave in the electroencephalograph (EEG).

Atypical absence seizures

Atypical absences are less common and usually occur in symptomatic epilepsies in children with pre-existing brain damage. These seizures are frequently associated with myoclonic activity.

1.1.2.4. Clonic seizures

Clonic seizures are characterized by rhythmic or semi-rhythmic muscle contractions, typically involving the upper extremities, neck and face.

1.1.2.5. Tonic seizures

This seizures cause sudden stiffening of extensor muscles, often associated with impaired consciousness and falling to the ground.

1.1.2.6. Atonic seizures

Atonic seizures produce sudden loss of muscle tone with instantaneous collapse, often resulting in facial or other injuries.

1.2 MECHANISMS OF EPILEPSY

Neuronal networks can be made to discharge abnormally by electrical stimulation, by alterations in basic metabolic environment as well as by application of specific neurotransmitters or drugs. Using such means it is possible to induce seizures in a "normal" brain. This has to be differentiated from the occurrence of spontaneous seizures in an altered network that is the characteristic of epilepsy.

1.2.1 Brain development and motor system

Certain regions may be considered seizure sensitive and have a low threshold and high susceptibility such as the motor cortex, the limbic structures and regions involved in autonomic function. The temporal lobe and its deeper limbic nuclear aggregates (amygdala) and hippocampus are particularly susceptible to seizure development. Age and development are also important factors from the perinatal period onward with a delay often noted between a specific brain insult and the later appearance of seizures.

1.2.2 Neuroanatomic and pathological changes

Hippocampal tissue removed during temporal lobe resection for intractable epilepsy demonstrates, in addition to the expected neuronal loss and gliosis, significant changes in dendritic complexity and organization (Thom et al, 2002; Billinton et al, 2001). This suggests that there are changes in connectivity and organisation of the neuronal network. The role that gliosis plays in the development of epilepsy is unknown; gliosis could play a part in the dysregulation of the neurochemical environment including "spill-over' of neurotransmitters and neurotransmitter accumulation. In addition to the anatomical changes there are also physiological changes including changes in receptors and channels that may all contribute to the epileptogenic state. These changes are not modified by our present antiepileptic treatments, which instead aim to redress neuronal and network dysfunction and dysregulation. To this end, specific targets for antiepileptic drugs have been identified.

1.2.3 Voltage-dependent membrane conductance

1.2.3.1 Voltage-sensitive sodium channels (VSSCs)

The VSSCs were originally found to be the molecular targets of specific AEDs (e.g. carbamazepine and phenytoin). These AEDs bind to the inactivated state of the VSSC, slowing the post-opening recovery phase. This effect may underlie the capability of these AEDs to prevent high frequency activity and reduce late VSSC-mediated sustained depolarisations that may contribute to seizure generation (Segal et

al, 1995). Genetic variation in splicing of a subunit of VSSCs may be predictive of the effective dose of antiepileptic drugs acting at this target (Tate et al, 2005).

1.2.3.2 Voltage-gated calcium channels (VGCCs)

There is considerable evidence available concerning the role of VGCCs in epileptogenesis. In particular genetic mutations in human calcium channel genes or those of several mouse species have been associated with epileptic phenotypes (Chen et al, 2005). The T-type VGCC has been implicated in playing a significant role in controlling epileptiform spike wave discharges associated with absence seizures (Gomora et al, 2001). There is evidence that both ethosuximide and valproate may act at this target, thus specifically modulating absence seizures (Coulter et al, 1989).

1.2.3.3 Voltage-gate potassium channels (VGPCs)

VGPCs have many key roles including setting resting membrane potential, determining firing threshold, action potential repolarization and determination of postsynaptic excitability (Errington et al, 2005). Therefore it would be expected that potentiation of potassium currents should have an antiepileptic effect. Madeja et al (2003) have suggested that paradoxically the antiepileptic properties of levetiracetam may be partly explained by a reduction in the conductance of delayed rectifier K+ channels. It has been reported lamotrigine is able to potentiate outward K_A conductance at concentrations similar to those at which they exert sodium channel inhibition (Zona et al, 2002). The functional diversity amongst the K+ channels makes them prospective targets for future AED development.

1.2.4 Synaptic mechanisms

1.2.4.1 Introduction

Epilepsy has been proposed to be associated with excessive stimulated release of glutamate and /or a failure of GABA modulated inhibition (van Gelder & Bowers, 2001). GABA and glutamate are undoubtedly involved in the mechanisms of epilepsy. However, GABA and glutamate are not the only amino acid neurotransmitters related to epilepsy, others have also been implicated. These include the excitatory transmitter aspartate, and the inhibitory transmitters glycine and taurine (Borgeat, 1997; Sveinbjomsdottir et al, 1993). This chapter will focus on these five amino acid neurotransmitters, There are other neurotransmitters such as serotonin, dopamine, acetylcholine and noradrenalaine, and peptides such as NPY, galanin and subtance P, which play important roles in the regulation of cortical excitability, but which do not form a part of this thesis.

1.2.4.2 Inhibitory mechanisms

GABA

GABA has had a central role in neural control theory since it was first discovered in 1950 (Roberts & Frenkel, 1950). GABA was later shown to inhibit seizure activity after its direct cerebral application (Meldrum, 1978). This gave rise to the hypothesis that GABAergic inhibition is an important factor in the suppression of seizure activity in epileptic patients.

1. GABA synthesis and metabolism

The main pathway of GABA synthesis involves the decarboxylation of L-glutamate by glutamic acid decarboxylase (GAD) (Tina et al, 1999). The synthesis and metabolism of GABA associated with nerve terminals has been linked to a substrate cycle between neurons and astrocytes involving glutamate, GABA, and glutamine (Shank et al, 1993). In this cycle, nerve-terminal GABA is synthesized from glutamate and enters the ECF by neurotransmitter release; it is then either recycled into the nerve terminal or taken up by astrocytes (Figure 1.1).

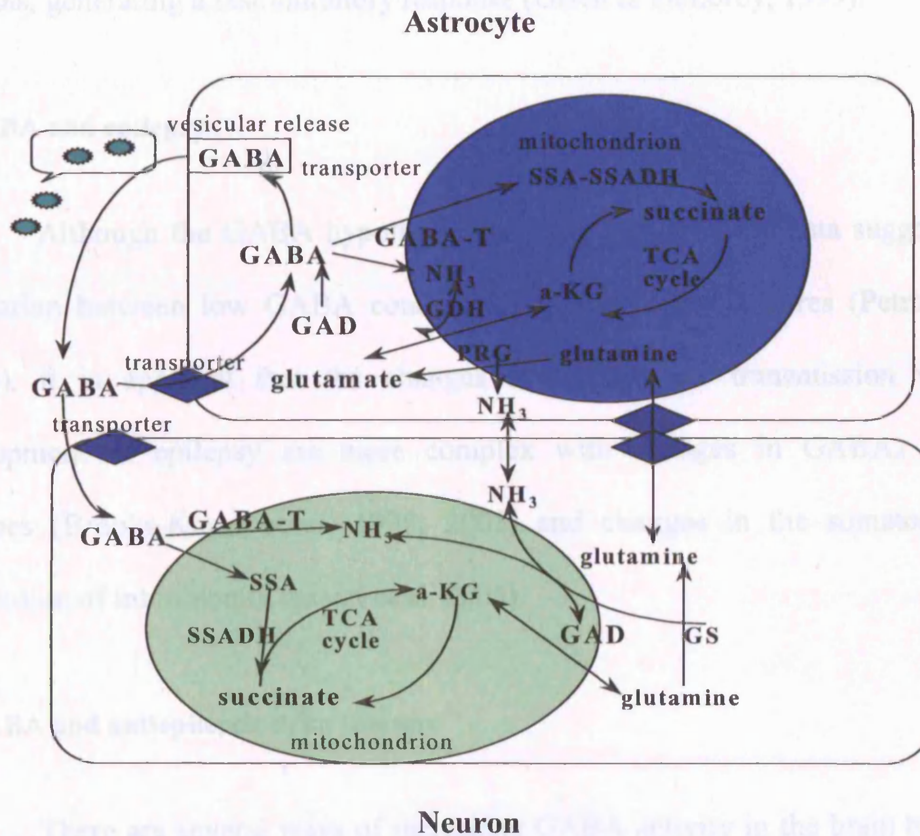


Figure 1.1: A schematic of the GABA synapse.

In astrocytes, GABA is broken down by GABA transaminase and resynthesized into glutamate. Astrocytic glutamate is converted into glutamine that is

taken up by nerve terminals. In the nerve terminal, the glutamine is hydrolyzed to glutamate by glutaminase (Olsen & DeLorey, 1999).

2. GABA receptors

GABAergic neurotransmission may be divided into slow and fast components. $GABA_B$ (metabotropic) receptors produce slow, long lasting inhibition since they are only indirectly coupled to calcium and potassium channels, via G-protein. $GABA_A$ and $GABA_C$ (ionotropic) receptors are directly coupled to chloride channels, generating a fast inhibitory response (Olsen & DeLorey, 1999).

3. GABA and epilepsy

Although the GABA hypothesis has been supported by data suggesting an association between low GABA concentrations and recent seizures (Petroff et al, 1996b), it is apparent that the changes in GABAergic transmission with the development of epilepsy are more complex with changes in $GABA_A$ receptor subtypes (Brooks-Kayal et al. 1998; 2001) and changes in the somatodendritic distribution of inhibition (Crossart et al, 2005).

4. GABA and antiepileptic drug therapy

There are several ways of increasing GABA activity in the brain by AEDs. (1) GABA agonists (e.g. progabide, diazepam and phenobarbital) directly increase inhibitory chloride conductances and up regulate the effect of synaptically released GABA on the $GABA_A$ receptors. (2) GABA transporter blockers (e.g. tiagabine) prolong the action of GABA in the synaptic cleft by inhibiting uptake and increase

extracellular GABA concentrations. (3) Stimulating GABA synthesis and release of synaptic GABA during neuronal activation. (4) Slowing degradation of GABA (e.g., vigabatrin) by inhibiting GABA transaminase (GABA-T), and increasing intracellular and extracellular GABA concentrations.

Glycine

Glycine is a key neurotransmitter in CNS. Two separate systems have been identified. The first one is an inhibitory function that is mediated by glycine receptors at the site of action of the convulsant strychnine. The second type of glycine recognition site is part of the NMDA receptor complex. Glycine potentiates the excitory action of glutamate (Tunnicliff, 2003).

1. Glycine synthesis and metabolism

Glycine is metabolically derived from serine by the action of D-serine-transhydroxylmethylase (Figure 1.2).

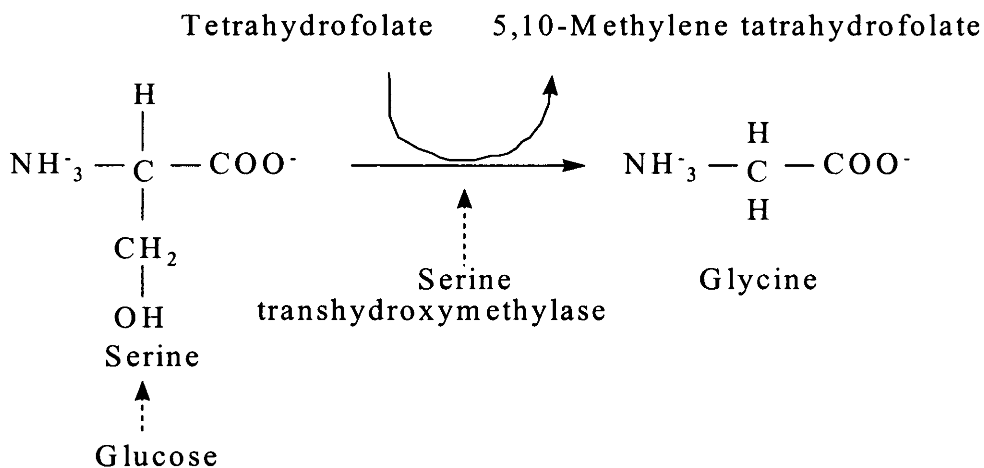


Figure 1.2: Glycine formation from serine by serine transhydroxymethylase

2. Glycine receptors

Glycine receptors are formed either from α subunts alone, or from both α and β subunits. Heteromeric glycine receptors have subunit composition $\alpha 3\beta 2$ (9-11) and three agonist-binding sites, rather than the two sites found in nicotinic acetylcholine or $GABA_A$ receptors.

3. Glycine and epilepsy

Glycinergic inhibition occurs primarily in the spinal cord and brain stem where it mediates fast inhibitory synaptic transmission via chloride permeable glycine receptors. In audiogenic seizure-susceptible rats, glycine levels were significantly decreased in the hippocampus and pons after habituation was obtained by stimulations repeated at 2 minutes interval. The mechanism for this is unknown (Hategan et al, 1990).

The involvement of glycine in human epilepsy is less clear and the few studies were rather contradictory. Crawford & Chadwick (1987) investigated the amino acid changes in CSF from 69 epileptic and 51 control patients. They found that glycine concentrations were significantly reduced in the patients CSF with epilepsy compared to the CSF from healthy controls. In contrast, Honda (1984) found that CSF glycine concentrations had increased in children with convulsive disorders. In surgical patients, glycine concentration is significantly increased in epileptogenic cerebral cortex (Sherwin, 1999). When glycine was administered in combination with phenobarbital or carbamazepine, glycine significantly enhanced the anticonvulsant potency of phenobarbital and carbamazepine. Glycine also potentiated the anticonvulsant actions of MK-801 and diazepam in the model of maximal

electroshock seizures (Peterson et al, 1990). These findings suggested a possible interaction between glycine and anticonvulsant drugs at NMDA receptors, the mechanism of which is unclear.

4. Glycine and epileptic therapy

There is no glycine receptor agonist available for treating epilepsy. Glycine antagonists MDL 100,458 and MDL 102,288 had different efficacies in the DBA/2 mouse model, although both substances showed comparable results in binding assays for the glycine binding site of the NMDA receptor. SM-319000, a glycine site antagonist with high selectively and affinity was shown to be anticonvulsant after systemic administration in NMDA-induced convulsions (Jansen & Dannhardt, 2003).

Taurine

The precise role of taurine in synaptic transmission is uncertain but its antiepileptic effects have been confirmed in several models of experimental epilepsy and in short-term clinical studies (Fariello et al, 1985; Lombardini, 1992; van Gelder, 1992; Richards et al, 1995). Several clinical trials involving taurine supplementation in epileptic patients have been reported and variable success has been achieved (Timothy & Birdsall, 1998). The mechanisms of action of taurine in the treatment epilepsy may be via binding to GABA or to glycine receptors, but it still lacks unique population of receptor sites (Gupta et al, 2005). Taurine modulates release of GABA but their bio-chemical action is different. Similarly, taurine and glycine have several similar activities but they work on a different receptor. How taurine concentrations in

the ECF relate to epilepsy onset and the relationship between its mechanism of action and efficacy are still unknown.

1.2.4.3 Excitatory mechanisms

The two mainly excitatory amino acids, namely glutamate and aspartate are known to act as agonists at all subtypes of ionotropic glutamate receptors. These excitatory amino acids play an important role in burst generation, and an integral part in the generation and spread of seizures.

Glutamate

Glutamate's excitatory potential was first noted in 1951 following intracarotid injections of glutamate in the study of epileptic phenomena (Okamoto, 1951). A year later, Hayashi (1952) injected small amounts of glutamate directly into the cortex, which demonstrated that a local application of glutamate could induce the firing in rat neocortical neurones of the rat brain. After the 1980's, the role of glutamate as the major cause of seizure mediated neuronal damage was studied with particular emphasise on the NMDA receptors.

1. Glutamate synthesis and metabolism

Glutamine and α-ketoglutarate are thought to be the major precursors for glutamate. Glutamine is taken up into the presynaptic terminal via an active, Na^+-dependent uptake protein. The glutamate in the terminal is then actively taken up into vesicles for future release into the cleft. From the cleft, glutamate is either actively taken back up via glutamate transporters or diffuses form the cleft and is taken up by

glutamate transporters on glia. Once in glial cells, glutamate is metabolized via glutamine synthase into glutamine or α-ketoglutarate. Glutamine is transported out of the glial cells and back into the presynaptic neurone for subsequent resynthesis of glutamate (Danbolt, 2001) (Figure:1.3).

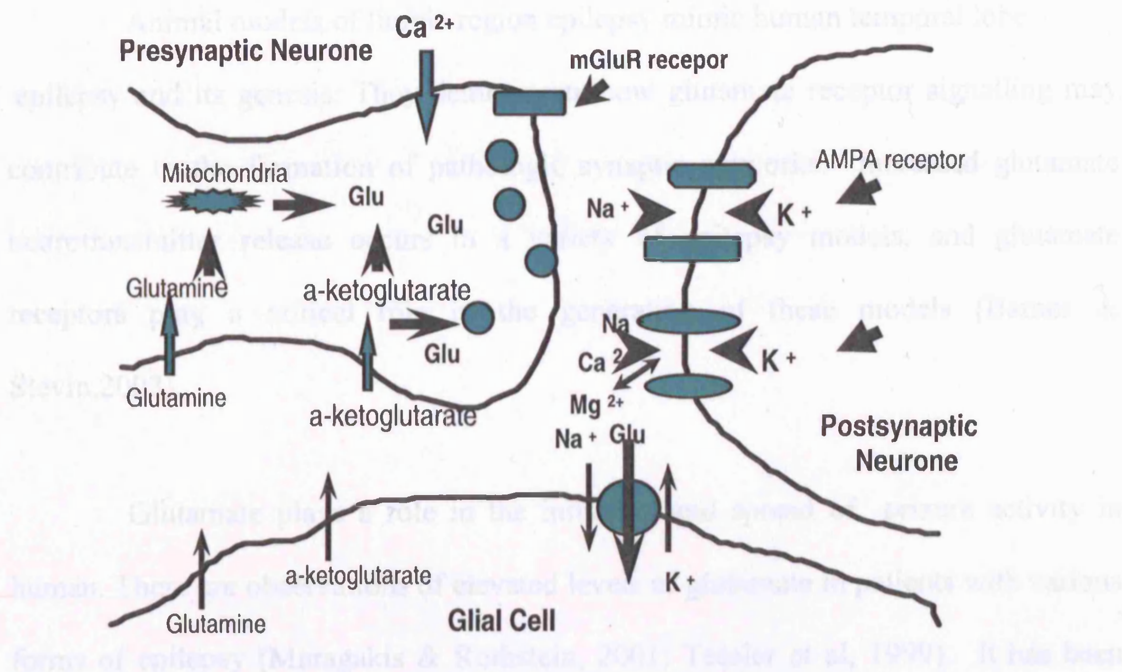


Figure 1.3: Schematic diagram of a model excitatory synapse

2. Glutamatergic receptors

Glutamate receptors include ionotropic and metabotropic receptors. The ionotropic receptors are further divided into three types - AMPA, kainate and NMDA receptors. The AMPA and kainate receptors are thought to mediate rapid depolarizing responses at most synapses in the mammalian CNS (Lerma et al, 1997). NMDA receptor activation contributes to diverse physiological responses, including synaptic formation and plasticity (Kirkwood et al, 1995). The metabotropic

receptors consist of at least eight different subtypes which are coupled to intracellular enzymes and to ion channels (Robinson, 1999).

3. Glutamate and epilepsy

Animal models of limbic region epilepsy mimic human temporal lobe epilepsy and its genesis. They demonstrate how glutamate receptor signalling may contribute to the formation of pathologic synaptic networks. Increased glutamate neurotransmitter release occurs in a variety of epilepsy models, and glutamate receptors play a critical role in the generation of these models (Barnes & Slevin,2003).

Glutamate plays a role in the initiation and spread of seizure activity in human. There are observations of elevated levels of glutamate in patients with various forms of epilepsy (Maragakis & Rothstein, 2001; Tessler et al, 1999). It has been suggested that a defect of uptake might be responsible for increases in glutamate concentration seen in the brain of patients with various forms of epilepsy (Barnes et al, 2003; Giger et al, 2000).

4. Glutamate and antiepileptic drug therapy

Although glutamate receptor antagonism would seem an ideal approach to the development of antiepileptic drugs, specific antagonists have not become established in clinical practice (often due to unacceptable side-effects). Nevertheless some antiepileptic drugs may have an action at glutamate receptors; these include topiramate at AMPA receptors and felbamate at NMDA receptors.

1.3 GENERAL PRINCIPLES OF NEW ANTIEPILEPTIC DRUGS

1.3.1 Mechanisms of action of new antiepileptic drugs

In general, new AEDs appear to decrease neuronal excitability or to enhance inhibition by altering sodium, potassium or calcium conductances or by affecting the activity of GABA, glutamate, or other neurotransmitters that may be involved in seizures. Although several AEDs share common mechanisms, each has distinct actions. Many new AEDs have mechanisms of action different from those of the more established agents (see Figure 1.4, LaRoche & Helmers, 2004).

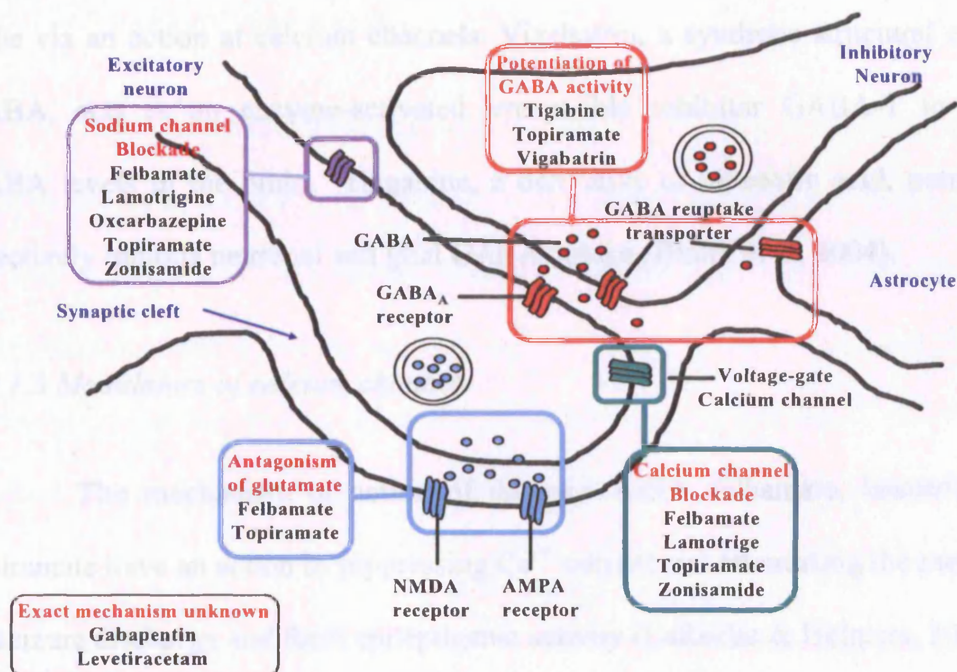


Figure 1.4: The principal mechanisms of action of the new AEDs.

1.3.1.1 Modulators of voltage-gate sodium channels

The voltage-gated sodium channel is involved in the generation and propagation of Na^+-dependent action potentials, and delaying its reactivation

significantly limits the frequency of neuronal firing. Like carbamazepine and phenytoin, the new AEDs oxcarbazepine, lamotrigine, felbamate and topiramate act via modulating the voltage-gate of sodium channel (LaRoche & Helmers, 2004).

1.3.1.2 Modulators of GABAergic inhibition

Although the mechanism of action of the new AEDs vigabatrin, gabapentin and tiagabine are different, they commonly enhance GABAergic inhibition. Gabapentin is a cyclic GABA analog designed to mimic a restricted static conformation of GABA with high lipid solubility for penetration of BBB, initially intended to act as a GABA agonist. Its mechanism of action, however, mainly seems to be via an action at calcium channels. Vigabatrin, a synthetic structural analog of GABA, acts as an enzyme-activated irreversible inhibitor GABA-T to increase GABA levels in the brain. Tiagabine, a derivative of nipecotic acid, potently and selectively inhibits neuronal and glial GABA uptake (Bialer et al, 2004).

1.3.1.3 Modulators of calcium channels

The mechanism of action of the new AEDs felbamate, lamotrigine and topiramate have an action in suppressing Ca^{++} current and attenuating the propagation of seizure discharge and focal epileptigenic activity (LaRoche & Helmers, 2004).

1.3.1.4 Other mechanisms

Felbamate has multiple mechanism of action. It acts at the $GABA_A$ receptor and may also have an antagonism effect on glutamate (LaRoche & Helmers, 2004). Levetiracetam's mechanisms of action are still largely unknown. There is some

evidence to suggest that synaptic vesicle protein (SV2A) is the binding site for levetiracetam, which suggest the protein involved in mechanism of levetiracetam (Lynch et al, 2004).

1.3.2 Principles of pharmacokinetics of new antiepileptic drugs

The pharmacokinetics for intraperitoneal administration (I.P.) is similar in pattern to that obtained after intravenous (I.V.) administration. The experiments in this thesis used I.P. administration. The concepts of clearance (*Cl*), extraction ratio, volume of distribution (*V*d) and half-life ($t_{1/2}$) are described in this section.

1.3.2.1 One-compartment model

When drug distribution is rapid compared with drug elimination, the entire body mass can be treated mathematically as a single compartment (one compartment) of volume (Fig. 1.5). The one-compartment can be seen after I.V., I.P. or S.C. administrations and refers to the fact that immediately after administration, drug concentration at the site of reference (blood or plasma) declines over time in an apparent monoexponential fashion with no visible distribution phase.

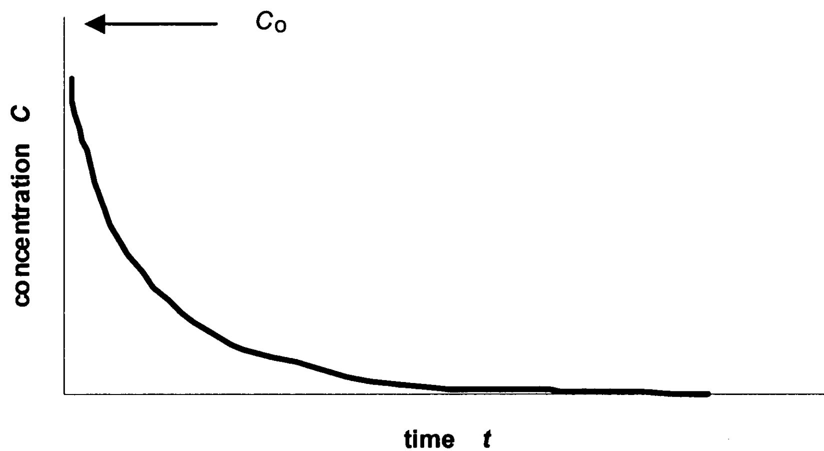


Figure 1.5: The time course of drug in blood following a single I.P. or I.V. dose in one-compartment model. The solid line represents the line drawn through a series of plasma concentration determinations.

1.3.2.2 Half-life

$T_{1/2}$ is defined as the time required for blood drug concentration to decrease by 50%. This relationship shows that the larger the elimination rate constant, the shorter the half-life.

$$T_{½} = 0.693/K$$

1.3.2.3 Area under curve

Area under curve (AUC) is the pharmacokinetic parameter that closely related to the clearance (*Cl*) of a drug. A measure of this area can be a useful index of the biological availability of the drug and a means of estimating its *Cl*. Figure 1.6 shows how AUC is calculated using the trapezium rule.

$$\text{AUC} = \frac{(a + b) \times (t1 - t0)}{2}$$

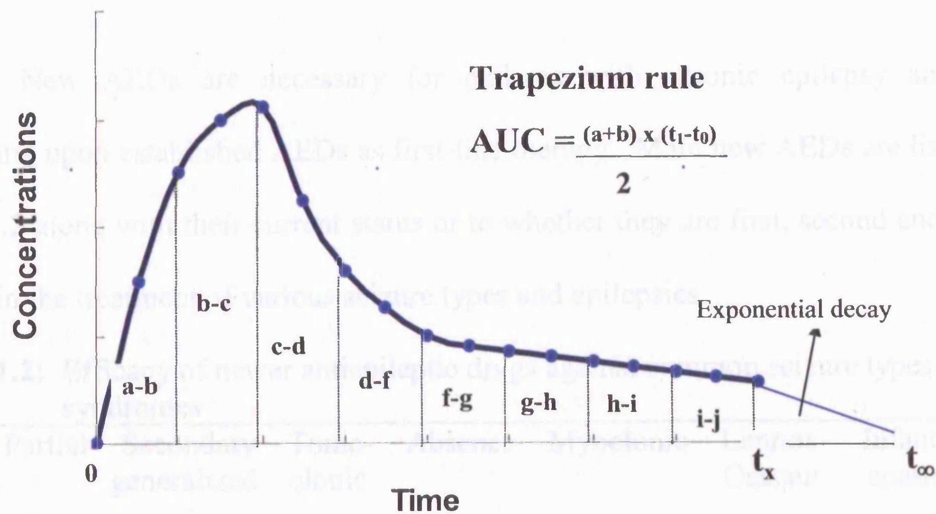


Figure 1.6: Graph showing how AUC is calculated using the trapezium rule.

1.3.2.4 Drug distribution and elimination

When drug is administered as a single bolus dose, the initial concentration in the body has a value *Co*. The amount (A) of drug in the body is *Ao* and a volume of distribution (*V*d) can be determined by the ratios.

$$Vd = D/Co = A/C$$

For drugs exhibiting one-component compartment behaviour, this *V*d is also the ratio of drug amount in the body to blood drug concentration (C) at any time after administration.

Clearance (*Cl*) is the apparent volume of blood completely cleared of drug per unit time. If pharmacokinetics is linear, clearance is dependent of dose.

$$Cl = Dose/AUC$$

1.3.3. Principles of new AEDs therapy

New AEDs are necessary for patients with chronic epilepsy and for improving upon established AEDs as first-line therapy. Main new AEDs are listed in Table 1.2 along with their current status or to whether they are first, second and third choice in the treatment of various seizure types and epilepsies.

Table 1.2: Efficacy of newer antiepileptic drugs against common seizure types and syndromes

	Partial	Secondary generalized	Tonic-clonic	Absence	Myoclonic	Lennox-Gastaut	Infantile spasms
FBM	+	+	?+	?+	?	+	?
GBP	+	+	?+	-	-	?	?
LEV	+	+	+	?+	+	?	?
LTG	+	+	+	+	+*	+	?+
OXC	+	+	+	-	-	0	0
PGB	+	+	?	?	?	?	?
TGB	+	+	?	-	?	?	?+
TPM	+	+	+	?	+	+	?+
VGB	+	+	?+	-	-	?	+
ZNS	+	+	+	?+	+	?+	?+

* Lamotrigine may worsen myoclonic seiszures in some cases
\+ proven efficacy; **?+** probable efficacy; **0** ineffective; **-** worsens control;
? unknown. (Brodie et al, 2005)

1.4 DRUG INVESTIGATED IN THE THESIS

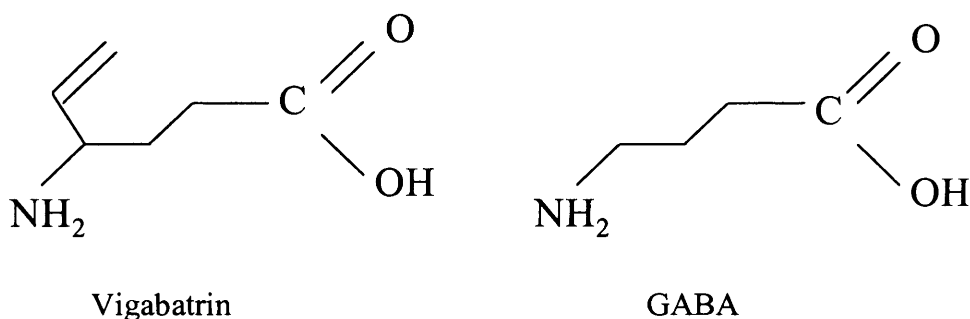
In this thesis, two new AEDs, vigabatrin and levetiracetam, were investigated in relation to their pharmacokinetics and neuropharmacokinetics

characteristics, and also their effects on brain neurotransmitters and electrophysiological properties. Levetiracetam is a new AED and has been licensed in Europe and American. Its mechanisms of action have not been completely elucidated. In contrast, vigabatrin is an AED drug with a well estrablished mechanism of action - inhibiting GABA-T which decreases GABA breakdown and increases brain GABA concentrations. This thesis aims to investiage and comparethe pharmacokinetics, neurophamacokinetics and mechanisms of these two drugs.

1.4.1 Vigabatrin

1.4.1.1 Chemistry

Vigabatrin, 4-amino-hex-5-enoic acid; gamma-vinyl GABA, is a structural analogue of GABA (Figure 1.7) and it is freely soluble in water (pH value 4.02 and 9.72). Presently it is supplied as a racemic mixture of its two enantiomers: the S(+) and R(-) enantiomers. Only the S(+)-enantiomer is pharmacologically active. The R(-)-enantiomer does not interfere, and neither undergoes *in vivo* inversion (Haegele & Schechter, 1986).



Vigabatrin GABA

Figure 1.7: Chemical structure of vigabatrin and GABA (Richens, 1989)

1.4.1.2 Pharmacology

Mechanism of action

Vigabatrin, as an analogue of GABA, acts by replacing GABA as a substrate of GABA-T. It irreversibly and covalently binds to the enzyme and enhances GABA-mediated inhibition by preventing GABA degradation (Angehagen et al, 2003). In an early study (Jung et al, 1977), vigabatrin was shown to produce dose-related inhibition of GABA-T in mice. They injected vigabatrin to male mice intraperitoneally with increasing doses. Eight hours post treatment, GABA concentrations, GABA-T, and GAD activities were measured in the brain. GABA-T activity decreased in a dose-dependent manner. Even at the smallest vigabatrin dose (100 mg/kg) a 45 % reduction in GABA-T activity was found.

Changes in CSF GABA and other neurotransmitters in humans have been observed after vigabatrin therapy (Grove et al, 1981; Ben-Menachem et al, 1989; Rothman et al, 1993; Mattson et al, 1994). Increasing vigabatrin dosage from 3g/day to 6g/day did not result in a further increase in brain GABA concentrations (Rothman, et al, 1994). Vigabatrin 50 mg/kg/day as add-on therapy was given to 11 patients with drug-resistant complex partial seizures as a single-dose, every third day, every other day and daily (Ben-Menachem et al, 1989). Serial lumbar punctures taken pre- and post treatment revealed that total GABA, free GABA and homocarnosine concentrations increased significantly in a dose-dependent manner.

NMR spectroscopy of epileptic patients treated with vigabatrin in addition to conventional AEDs has demonstrated increased brain GABA concentrations

consequent to vigabatrin therapy (Petroff et al, 1995). The authors also found increased levels of glutamine and decreased levels of glutamate by 9% compared to other patients on conventional AED therapy alone. In another study, CSF GABA increases were correlated with decreases in regional cerebral metabolism determined by PET studies (Spanaki et al, 1999). Although originally considered not to have an effect on GABA uptake (Schousboe et al, 1986), evidence now suggests that vigabatrin may in part act through this mechanism (Jolkkonen et al, 1992).

Anticonvulsant activity in seizure models

The anticonvulsant effects of vigabatrin have been observed in a variety of experimental models of epilepsy. It is effective against seizures induced by strychnine, pentyleneterazole, electroshock, methionine sulphoxide, picrotoxin, isoniazid and ethionine and mercaptopropionic acid (Miller et al, 1987; Tossi et al, 1987; Bernasconi et al, 1988; Bonhaus & McNamara, 1988; Sarhan et al, 1984; Sayin et al, 1993). It is also effective in genetically susceptible seizure models such as autogenic DBA/2 mice, hyperthermia-induced chicks and in photoepileptic baboons (Schechter et al, 1977; Meldrum & Horton, 1973). The drug has both antiepileptogenic and anti-seizure properties in the kindling model (Shin et al, 1986; Stevens et al, 1988). Vigabatrin has also been shown to protect hippocampal structures and function in the perforant pathway stimulation model of epilepsy (Ylinen et al, 1991).

Anticonvulsant action in epileptic patients

Vigabatrin is used as first line AED for treatment of infantile spasms (Hancock et al, 2003; Riikonen, 2005). In a randomized study in UK, vigabatrin was effective in 54% of the children. The difference in favour of hormonal treatment on spasms (70%) was significant at day 14 (Lux et al, 2004). There is evidence to suggest that vigabatrin is the treatment of choice in patients with tuberous sclerosis with infantile spasms, with a mean response rate of 74% in eight studies (Riikonen, 2004). The numbers of patients who were seizure-free at 3-4 months after ACTH or vigabatrin treatment are very similar (Riikonen, 2005).

Vigabatrin was also used in complex partial seizures. The early six double blind, placebo-controlled, crossover studies were conducted between 1984 and 1987. Patients with partial seizures were given a dosage range from 2-3g/day of vigabatrin as add-on therapy to other AEDs. All six studies showed a significant improvement in seizure frequency (Rimmer & Richens, 1984; Gram et al, 1985; Remy et al, 1986; Tassinari et al, 1987). Currently, the observation that vigabatrin causes visual field defects in at least a third of patients has led to a restriction in its use. Therefore, vigabatrin is not being used in America, but remains in EU market on a provisional basis. The approved indications have been restricted to partial epilepsy when all other appropriate drug combinations have proven inadequate or have not been tolerated (Bialer et al, 2004).

1.4.1.3 Pharmacokinetics

Absorption

Vigabatrin shows rapid absorption in both animal model and patient studies. In animal models, T_{max} of vigabatrin was about 1 hour in mice (Jung et al, 1977). Plasma AUCs of vigabatrin were similar in i.v., i.p. and oral administrations (Patsalos & Duncan, 1995). T_{max} for the dog was 5 minutes and 1 hour for I.V. and oral respectively. Comparison to oral and I.V. AUC values in dogs suggested that oral bioavailability of vigabatrin was high (Smithers et al, 1985).

Table 1.3 lists the key pharmacokinetic characteristics of vigabatrin in man. In a single-dose kinetic study, vigabatrin was determined for both enantiomers (Haegele & Schechter, 1986). Peak plasma concentrations for both enantiomers were reached at between 0.5 and 2 hours after a 1,500 mg dose. The concentration of the two enantiomers did not differ after 24 hours. The absorption was rapid, with the peak concentration reached in the first 2 hours after dosing of between 0.5-3 g. Elimination half-life ranges from 5 to 7 hours and vigabatrin was still detectable in the plasma after 24 hours. Food ingestion does not affect either the rate or the amount of vigabatrin absorbed (Frisk-Holmberg et al, 1989). The kinetics of multiple dose vigabatrin (1.0-4.0 g daily) was consistent with that of single dose acute administration (Hoke et al, 1993). There appeared to be no significant difference in kinetics when healthy volunteers and patients with epilepsy were compared. The bioavailability of vigabatrin has not been ascertained in man in absolute terms, since an I.V. formulation is not available for clinical use. However, it has recently been confirmed that 80-90% of an oral dose of vigabatrin is recovered in the urine of

healthy volunteers (Durham et al, 1993), suggesting that the bioavailability of vigabatrin is at least 80-90%.

Table 1.3: Vigabatrin Pharmacokinetics in patients

T_{max}	1-4 hours (unaffected by food)
$t_{1/2}$	5-7 hours
Protein binding	0%
Elimination	70 % renal
Vd	0.8l/kg
Active metabolites	None
Drug interaction	Minimal (reduce phenytoin levels?)
Liver enzyme induction	No
Accumulation	No

(Ben-Menachem, 1995)

Distribution

Only limited data have been reported as to the distribution of vigabatrin in animals. The unpublished data of distribution into various body tissues of rats, using radiolabel vigabatrin showed that vigabatrin was widely distributed and that distribution occurred rapidly with a half-life of distribution at 1-2 hour (Patsalos & Duncan, 1995).

The *Vd* of vigabatrin in patients is 0.8 l/kg (Table 1.5), which is similar to that of total body water 0.6 l/kg (Schechter, 1989). Vigabatrin is not protein-bound and since it is a highly water-soluble compound, a wide distribution in the body is observed. The tissue distribution of vigabatrin has not been investigated in man.

Elimination and metabolism

There was no report of elimination and metabolism of vigabatrin in animals. In epileptic patients, vigabatrin is minimally metabolised. It did not affect hepatic microsomal enzyme activity (Bartoli et al, 1997) and is excreted as unchanged drug in urine with a clearance similar to that of glomerular filtration rate (Durham et al, 1993). Elimination was not dose-dependent at doses of 0.5-4.0 g and followed first-order kinetics (Grove et al, 1984; Hoke et al, 1993). In healthy volunteers, the values for elimination half-life and renal clearance, of both enantiomers, were 5-7 hours and 1.3 ml/min/kg, respectively (Haegele & Schechter, 1986; Saletu et al, 1986; Frisk-Holmberg et al, 1989). Only two metabolites (a lactam conjugate) of vigabatrin have been detected in the urine of man and these represent less than 5% of the total vigabatrin dose (Durham et al, 1993).

1.4.1.4 Neuropharmacokinetics

Jung and his colleges were the first time to investigate vigabatrin concentration in the mouse brain (1977). Vigabatrin rapidly entered the brain following I.P. administration. The peak plasma concentrations of vigabatrin were reached (30 ± 1.4 μg/g) within one hour, concentrations then declined according to first order kinetics. The half-life of vigabatrin in the brain was 16-17 hours and it was still detectable 3 days late. Bernasconi et al (1988) also showed the T_{max} of vigabatrin in rat CSF and the brain were achieved within 0.8 and 1 hour respectively.

The neuropharmacokinetics of vigabatrin in patients have only been studied in the CSF. An early study was carried out by Grove et al (1981). Patients with

various neurologic conditions received vigabatrin 0.5, 1, 2 or 6 g daily for 3 days. CSF concentrations of vigabatrin increased in a dose-response manner. Ben-Menachem (1989) reported that vigabatrin readily entered the CNS of patient with epilepsy, CSF concentrations of vigabatrin was approximately 10-15 % of blood concentration. Maximum vigabatrin concentration (11.5μmol/L) in CSF occurred at 6 hours after oral ingestion of 50 mg/kg vigabatrin (Ben-Menachem et al, 1988). Patients given repeated doses of vigabatrin exhibited substantial variability in CSF vigabatrin concentrations at 24 hours post-dose. Vigabatrin was not detectable at 72 hours (Ben-Menachem et al, 1989).

1.4.1.5 Effect on amino acids

GABA

Vigabatrin increases brain GABA concentrations by inhibiting GABA-T at presynaptic neurones (see Figure 1.8). June et al (1977) was the first to investigate this effect in a mouse model. Vigabatrin 1500mg/kg was administered by I.P., and whole mice brain GABA concentration was increased following a decrease in GABA-T.

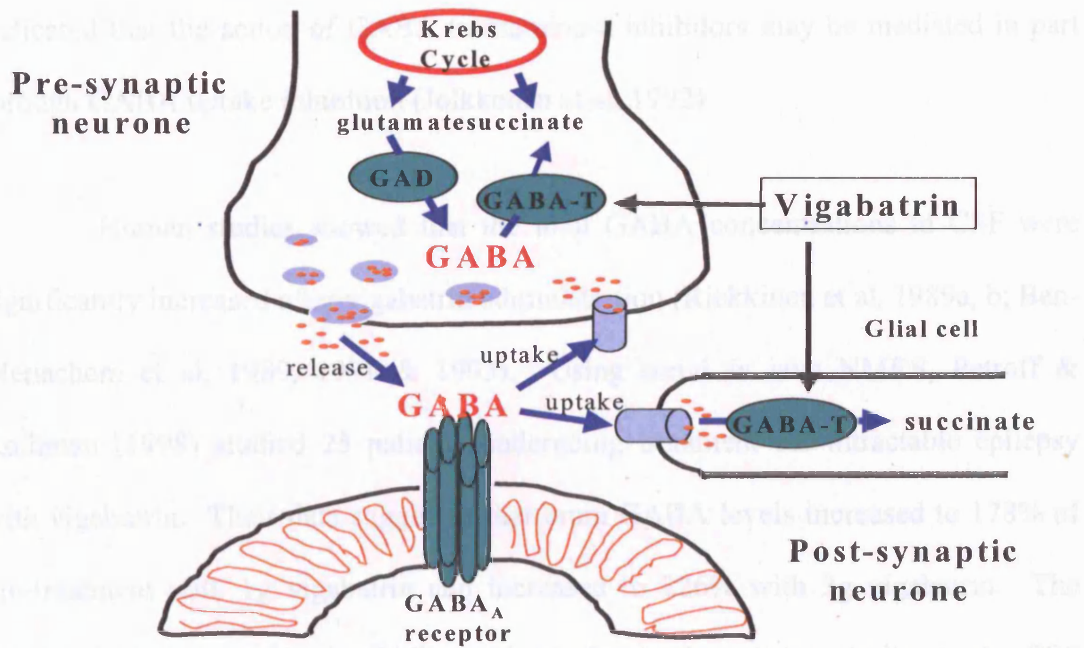


Figure 1.8: Synthesis, release, action at the $GABA_A$ receptor, and re-uptake of GABA after vigabatrin administration. Vigabatrin acts by destroying GABA-T, the enzyme responsible for the breakdown of GABA. The resultant increased in GABA available for release into the synaptic cleft increases the probability of binding to $GABA_A$ receptor

The effect of vigabatrtin on extracellular GABA content was also investigated by microdialysis (Sayin et al, 1995). It was observed that vigabatrin administration induced a 4-6-fold increase in the extracellular content of GABA after 1000mg/kg administration. This increase lasted for at lease 72 hours. In the hippocampus, GABA concentration was monitored in rat after two doses of vigabatrin (1.6 and 8.0 mM) perfusion via the microdialysis probes . It was observed that vigabatrin caused a transient increase in the basal GABA outflow (10 fold) during the period of drug perfusion. The immediate increase of GABA outflow after vigabatrin may be due to a direct blockade of GABA uptake sites. These findings

indicated that the action of GABA transaminase inhibitors may be mediated in part through GABA uptake inhibition (Jolkkonen et al, 1992)

Human studies showed that the total GABA concentrations in CSF were significantly increased after vigabatrin administration (Riekkinen et al, 1989a, b; Ben-Menachem et al, 1989, 1991 & 1993). Using serial *in vivo* NMRS, Petroff & Rothman (1998) studied 25 patients undergoing treatment for intractable epilepsy with vigabatrin. Their data suggested that brain GABA levels increased to 178% of pre-treatment with 1g vigabatrin and increased to 226% with 3g vigabatrin. The fractional elevation of brain GABA with vigabatrin doses were similar to the CSF GABA increase suggesting a proportional relationship between intracellular and extracellular GABA concentrations.

The increase in GABA release after GABA-T inhibition has been now attributed to a Ca^{++}-independent process mediated through the reversal in the direction of GABA transports (Wu et al, 2001, 2003). Extracellular GABA depends on that of intracellular GABA, the elevated intracellular GABA due to GABA-T inhibition would generate a condition allowing for an increase in the reversal of GABA transporters, leading to increased transport-mediated GABA release (Chen et al, 2005).

Glycine

The potential anticonvulsant effect of glycine have been reported in two generalized convulsive models early (Toth et al, 1983). In these models, vigabatrin and glycine was administered in combination and seizures were suppressed. The

results showed that when glycine was given alone, it provided partial protection against convulsive seizures. While the treatment of glycine combined with vigabatrin significantly suppressed seizures and enhanced the aggravating effect of vigabatrin on spontaneous spike and wave discharges in these models.

In epileptic patients, Pitkanen et al (1988) showed that glycine concentrations in CSF were increased by 128% after vigabatrin administration, but they suggest that this effect was not necessarily related to the clinical response. Glycine concentration was also significantly increased in epileptogenic cerebral cortex. These patients exhibited a two-fold increase in mean glycine concentrations after vigabatrin treatment (Sherwin, 1999).

Homocarnosine

Vigabatrin increase in central homocarnosine concentrations in both brain and CSF have been reported in many studies in animal models (Bohlem et al, 1980; Palfreyman et al, 1980, 1983; Halonen et al, 1990). Total conjugated GABA and homocarnosine elevation parallel the free GABA increase in CSF and they occur in relatively high concentrations and can be assayed more easily than free GABA in human CSF. Therefore, measuring conjugated GABA and homocarnosine were more reliable clinical indicators of brain free GABA concentration (Pikanen et al, 1988; Petroff et al, 1999).

In patients treated with vigabatrin, a dose-related increase in both free and total GABA and homocarnosine concentrations in the CSF has been observed (Grove et al, 1981; Schechter et al, 1984; Pitkanen et al, 1988; Ben-Menachem, 1989;

Rothman et al, 1993; Mattson et al, 1994). Analysis of amino acid content of brain tissue from a patient who underwent left side temporal lobectomy after treatment with vigabatrin for 3-5 years, revealed a three-fold increase in total, free GABA and homocarnosine (Pitkanen et al, 1988; Halonen et al, 1988; Riekkinen et al, 1988).

NMRS studies also showed that homocarnosine and vigabatrin increased linearly with daily vigabatrin administration and seizure control improved with increasing CSF homocarnosine concentrations (Petroff et al, 1998 b and 1999; Simister et al, 2003). Daily low-dose (2g) vigabatrin increased both homocarnosine and GABA, and seizure control was associated with the higher homocarnosine concentrations. GABA and homocarnosine concentrations increased in proportion to a daily vigabatirn dose (Petroff et al, 1999). Similarly, CSF observed from patients with epilepsy showed that homocarnosine and vigabatrin increased linearly and that seizure control improved with increasing CSF homocarnosine (Petroff et al, 1998a). They gave a single oral dose of vigabatrin and the rate of GABA synthesis in human brain was estimated at 17% of the Krebs cycle rate. As the daily dose of vigabatrin was increased up to 3g, the fractional elevation of brain GABA was similar to the increase seen in CSF. Two important relationships were proposed by them. 1), Elevated CSF GABA and homocarnosine concentrations are an index of high brain GABA concentrations. 2), Elevated CSF and brain GABA and homocarnosine were associated with improved seizure control and that homocarnosine may serve as storage form of GABA, helping the GABAergic neurones control GABA concentration.

Glutamate and other amino acids

Vigabatrin has been reported to affect the concentrations of other neurotransmitters. However, the changes in glutamate, glutamine and other amino acids are somewhat controversial. Glutamate, glutamine, aspartate and alanine concentrations decreased and lysine concentration increased in cortical tissue after vigabatrin administration (Bernasconi et al, 1988). A dose-dependent decrease in rat whole brain tissue concentration of glutamate after I.P. vigabatrin administration has been described (Halonen et al, 1991; Smolders et al, 1997). After 21 days of 150mg/kg vigabatrin administration, glutamate concentration was also reduced in different areas of rat brain (Waniewski & Martin, 1995). Furthermore, vigabatrin caused a decrease in the brain content of the excitatory amino acids glutamate and increase GABA was reported in a microdialysis study (Benturquia et al, 2004). In contrast, Halonen et al (1990) reported that elevation of glycine and taurine content coincided with peak level of vigabatrin in rat brain. Glutamate concentrations were also increased but the increase occurred somewhat late when the effect of vigabatrin on GABAergic transmission had markedly diminished. However, glutamine, aspartate and taurine concentration were reported as unaffected.

A decrease in CSF glutamate concentrations has been described in the patients receiving vigabatrin as monotherapy (Kalviainen et al, 1993). Using [^{1}H] NMR spectroscopy with vigabatrin at dose of 3-6 g/day, glutamate decreased by 9% in a group of 14 patients with epilepsy (Petroff et al, 1995). These changes could already be detected in brain tissue at 2-5 hours after administration of vigabatrin. The concentration of glutamine in human CSF decreased significantly after a single dose

of vigabatrin (Ben-Menachem, 1989). However, some studies indicated that glycine, glutamate, glutamine, aspartate, asparagines and taurine concentrations did not change in patient CSF (Pikanen et al, 1988; Halonene et al, 1988).

1.4.2 Levetiracetam

Levetiracetam (Keppra ™, ucb LO59), (S) -α-ethyl-2-oxo-pyrrolidine acetamide) is a novel drug which not only has potent anticonvulsant properties (Gower et al, 1995; Margineanu & Wulfert, 1997; Loscher et al, 1998), but also displays a highly favourable therapeutic index. Adverse effects in animal models were minimal with mild sedation and muscle relaxation occurring at doses 50 to 100-fold higher than those associated with seizure protection (Gower et al, 1992; Klitgaard et al, 1998).

In humans, levetiracetam has been observed to possess significant efficacy, good tolerability and a desirable therapeutic index (Kasteleijn-nolst et al, 1996; Betts et al, 2000; Cereghino et al, 2000, Welty et al, 2002). It has recently been licensed for use as add-on therapy in patients with intractable partial epilepsy.

1.4.2.1 Chemistry

Levetiracetam, is the S-enantiomer of the ethyl analogue of piracetam, a widely used nootropic agent (Loscher & Schmidt, 1993). Its molecular weight is 170.21 and chemical structure is shown in Figure 1.9. Levetiracetam is highly soluble in water (104.0g/100mL). Only the S-enantiomer of levetiracetam has anticonvulsant effects.

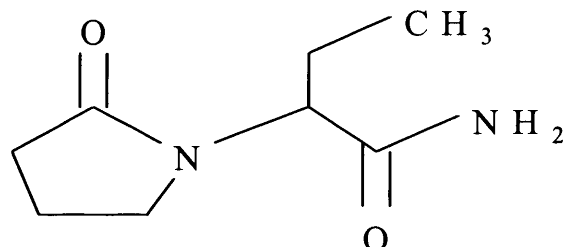


Figure 1.9: Chemical structure of levetiracetam (Patsalos, 2000)

1.4.2.2 Pharmacology

Mechanism of action

The precise mechanism of action of levetiratetam is not fully understood. Its action is independent of excitatory and inhibitory neuronal pathways (Nash & Sangha, 2001), and it failed to interact with classical receptors and ion channel sites in the brain (Noyer et al, 1995).

Recently it was shown that levetiracetam binds to SV2A, opening the new field of drugs acting on vesicle exocytosis (Lynch et al, 2004). SV2A is enriched in synaptic vesicles and has an apparent molecular mass of – 90 kDa. Brain membranes and purified synaptic vesicles from mice lacking SV2A did not bind a levetiracetam derivative, indicating that SV2A is necessary for levetiracetam binding. There was also strong correlation between the affinity of a compound for SV2A and its ability to protect against seizures in an audiogenic mouse animal model of epilepsy. The experimental results suggest that SV2A is the binding site for levetiracetam in the brain.

In brain synapses, nitric oxide (NO) synthase activation is coupled to NMDA-mediated calcium entry at postsynaptic densities through regulatory protein complexes, and NMDA/NO inhibit the release of pre-synaptic glutamate (Sequeira et al, 2001). The inhibitory retrograde action of postsynaptic NO on presynaptic terminals (Micheva et al, 2001) could contribute also to the effects of levetiracetam. The results (Dagonnier et al, 2005) confirm that NO should be considered as one of the key mediators involved in the effects of this growingly used medication for neurological disorders, including epilepsy. Madeja et al (2003) have suggested that the antiepileptic properties of levetiracetam may be substantially contributed to by a reduction in the conductance of delayed rectifier K+ channels. This study demonstrated using acute isolate rat and guinea pig CA1 neurones by whole cell patch clamp technique, that a 26% reduction in the delayed rectifier K+ current is produced by 100μM levetiracetam. (Madeja et al, 2003).

<u>Anticonvulsant action in seizure models</u>

Various rodent models were used for testing the anticonvulsant efficacy of levetiracetam *in vivo*. Levetiracetam lacks anticonvulsant activity in both MES and PTZ tests in rodents (Klitgaard et al, 1998). Other seizure paradigms in rodents involving threshold tests (Löscher, 1993) have shown only a weak anticonvulsant activity by levetiracetam (Gower et al, 1992).

However, levetiracetam is associated with a very potent protection against seizures in spontaneously epileptic rat (SER). The tonic convulsions and absence-like seizures in SERs were induced and the seizure activities were inhibited by 80, 160 mg/kg single dose of levetiracetem. When the drug was repeatedly administered at

80mg/kg for 5 days to SERs, the inhibitory effects on seizures increased with administration time. This long-lasting treatment effect of levetiracetam was not shared with other AEDs (Ji-qun et al, 2005). The long –lasting anti-seizure effects of levetiracetam have been also observed in SER in another study (Yan et al, 2005). Furthermore, seizure activity expression was prevented in both audiogenic susceptible mice. In contrast, other new AEDs are not so effective against these types of seizures (Leppik, 2001).

Anticonvulsant action in epileptic patients

The efficacy of levetiracetam as add on therapy has been assessed in partial seizure with refractory epilepsy. Three groups were tested in 904 patients (Shorvon & Rijckevorsel, 2002). The percentage reduction of seizure from baseline was 32.5 % for patient receiving levetiracetam treatment compared with 7% for patient's receving placebo. Cereghino and colleagues (2000) concluded that levetiracetam was effective and well tolerated as adjunctive therapy for partial-onset seizures, with or without secondary generalization. There are some indications that the drug would be useful in a wider range of seizure types and syndromes, these include SE (Rossetti & Bromfield, 2005), refractory epilepsy (Mohanraj et al, 2005; French et al, 2005), myoclonus (Lim & Ahmed, 2005) and different types of epilepsy in children (Vigevano, 2005; Mandelbaum et al, 2005). Levetiracetam has also been used as monotherapy for treating seizures. Alsaadi et al (2005) investigated levetiracetam monotherapy in. 46 patients with a history of partial seizures with and without secondarily generalization. Thirty-five patients have completed the study for a year. At the follow-up, nineteen of the 35 patients (54 %) were seizure free for past 6

months. Four patients (11.4%) had > 75% reduction in seizure frequency and nine patients had seizure reduction > 50%. This study suggests that levetiracetam as monotherapy can be effective and well tolerated in newly diagnosed epilepsy patients as well as for chronic epilepsy patients who failed multiple AEDs prior to levetiracetam treatment.

1.4.2.3. Pharmacokinetics

The pharmacokinetic profile of levetiracetam has been determined in a rat (Doheny et al, 1999), a dog (Isoherranen et al, 2001) and healthy volunteer studies (Shorvon & van Rijckevorsel, 2001; Nash & Sangha, 2001). Table 1.4 shows the pharmacokinetic parameters of levetiracetam following oral administration in patients.

Table 1.4: Pharmacokinetic profile of levetiracetam in humans following oral administration

Parameters	**Effect**
Absorption	Rapidly and completely (> 95%) *T*max 1.3 hr Food: rate of absorption slowed by 1.5 hr
Distribution	
Dose linearity	linear
Time to plasma steady-state	Approximately 48 hr
Plasma protein binding	0 %
Volume of distribution	0.5-0.7 L/kg
Elimination	
Half-life	Adults, 6-8 hr; elderly, 10-11hr; Children, 6 hr.
Metabolite	In urine: 66% as parent drug, 24% as UcbL057

(Nash & Sangha,2001; Patsalos 2003 & 2004)

Absorption

In the rat, levetiracetam rapidly appears in serum after I.P. administration at three doses (20, 40 and 80mg/kg). The concentration of levetiracetam rose linearly and dose-dependently, with mean peak concentration being achieved at 0.25-0.5 hours after levetiracetam administration (Doheny et al, 1999). In the dog, levetiracatam also quickly entered the plasma compartment and was detectable at time of first sampling after I.V. administration of 20mg/kg levetiracetam. Plasma C_{max} values were 100 and 40 mg/L for S-enantiomer and R-enaltiomer respectively (Isoherranen et al, 2001).

In patients, levetiracetam is rapidly and almost completely (> 95%) absorbed following oral administration of doses ranging from 250 to 5000 mg. Maximum plasma concentration of levetiracetam generally occurred within 1 hour. Absolute oral bioavailability was nearly 100% (Patsalos, 2000). Following oral ingestion, peak plasma concentrations (C_{max}) were achieved within 1 hour and then declined to baseline within 48 hours. Following a single 1000 mg levetiracetam daily dose and repeated 1000 mg twice daily doses, C_{max} values were typically 31 and 43μg/mL, respectively. In healthy volunteers, C_{max} and the AUC values displayed dose linearity in the range of 500-5000 mg. Steady-state concentrations were generally attained after 2 days of repeated twice-daily dosing. In multiple dose-ranging studies, levetiracetam exhibits predictable, linear, and dose-proportional steady-state pharmacokinetics. Levetiracetam was not affected by food in a study of 20 healthy male volunteers (Patsalos, 2000 & 2003).

Distribution

In the rat the *V*d of levetiracetam is 0.5-0.7 L/kg (Doheny et al, 1999). In the dog, the *V*ds of levetiracetam were calculated to be 0.45 ± 0.13L/kg and 0.51 ± 0.11 respectively for *S*- and *R*-enantiomers after I.V. administration of levetiracetam (Isoherranen et al, 2001). Distribution studies in the rat, mouse, rabbit and dog, showed that levetiracetam was rapidly distributed in tissue, with high concentrations occurring in blood and kidneys and with lower concentrations in the lens and adipose tissue (Patsalos, 2003).

Although tissue distribution data in humans are not available, levetiracetam is not bound to plasma protein. Thus, the risk for protein-binding displacement interactions being clinically significant is negligible.

Elimination and metabolism

The major route of excretion for levetiracetam is through urine. In rat models, levetiracetam elimination $t_{1/2}$ values are 1.8-3.0 hours (Loscher et al, 1998; Doheny et al, 1999), whilst in the dog $t_{1/2}$ values are 3.6-4.3 hours. *Cl* values for dog are 1.5 ml/min/kg (Isoherranen et al, 2001).

In healthy young volunteers, the elimination $t_{1/2}$ of levetiracetam was 6 to 8 hours, allowing a twice-daily dosing regimen (Patsalos et al, 2000; Perucca et al, 2003). Importantly, the elimination $t_{1/2}$ of levetiracetam was increased to 10-11 hours in elderly patients, and also was increased in patients with renal impairment (Patsalos, 2004).

Sixty-six percent of the administered dose is eliminated unchanged and 24% is excreted in urine as inactive metabolites (Patsalos, 2004). Excretion via the faecal route accounts for only 0.3% of the administered dose. Levetiracetam is cleared fairly rapidly, with 93% of drug excreted within 48 hours following oral administration. Renal clearance of levetiracetam occurs at a rate of 40ml/min/1.73 m^2 (0.6 ml/min/kg), which indicates excretion of levetiracetam by glomerular filtration and partial subsequent tubular re-absorption (Patsalos, 2000). Levetiracetam is metabolised by enzymatic hydrolysis of the acetamide group to one major metabolite, ucb L057; two minor metabolites have also been isolated (Nash & Sangha, 2001).

Drug-drug interaction

Evidence of the lack of significant influence of levetiracetam on the serum concentration of concurrently administered drugs is by the results of formal interaction studies *in vitro* and human. In vitro, levetiracetam, tested at therapeutically relevant concentrations, has been shown not to inhibit a variety of drug metabolizing enzymes, including cytochrome CYPs (Patsalos, 2000). In human, levetiracetam does not significantly affect steady-state serum concentrations of concomitantly administered carbamazepine, phenytoin, valproic acid, lamatrigine, gabapentin, phenobarbital, or primidone (Gidal et al, 2005). Levetiracetam was also equally found not to affect the pharmacokinetic profiles of digoxin (Levy et al, 2001), S-warfarin (Ragueneau-Majlessi et al, 2001), and steroid oral contraceptives (Ragueneau-Majlessi et al, 2001).

1.4.2.4 Neuropharmacokinetics

Although brain tissue distribution data in humans are not available, studies conducted in rats indicate that levetiracetam readily crossed the BBB and rapidly enters CSF (Doheny et al, 1999). T_{max} values for the CSF compartment were 1.3 – 1.9 hours. The dose-dependent increased levetiracetam in serum and CSF concentrations after levetiracetam administration (20, 40 and 80 mg/kg), suggest that transport across the BBB is not rate limiting over the concentration range observed (Doheny et al, 1999). The efflux of levetiracetam from the CSF compartment was significantly slower (mean $t_{1/2}$ 4.4 to 4.9 hours) than that from serum (mean $t_{1/2}$ 1.8 to 2.8 hours), and confirms similar observations with some other AEDs (Sokomba et al, 1988; Semba et al, 1993; Lolin et al, 1994; Walker et al, 1998).

1.4.2.5 Effect on amino acids

Whilst there were only a few reports of the effect of levetiracetam on amino acids in animal models, there are no studies describing such effects in humans. Margineanu & Wulfert (1997) have confirmed that there was no direct effect of levetiracetam on GABA receptor or brain GABA concentrations. Using classical labelled amino acids as radioligands to assess the binding sites of levetiracetam, Noyer et al (1995) showed no significant affinity by levetiracetam for known GABA, benzodiazepine or various excitatory amino acid-related receptors. Gower et al (1994) have confirmed that levetiracetam did not directly interact with the benzodiazepine-GABA ionophore. Furthermore, levetiracetam failed to influence the concentration of GABA, glutamate and glutamine in mouse brain following both single and repeated administrations of levetiracetam. These results suggest that

levetiracetam does not have any direct effect on GABA metabolism nor does it interact with GABA receptors (Welty et al, 2002). Another study (Rigo et al, 2000) evaluating the effect of levetiracetam on GABA- and glycine-gated neuronal currents, showed that levetiracetam was devoid of any effects. In contrast to other AEDs, minimal effects were observed on GABA-gated neuronal currents and levetiracetam had no effects on glutamate (Hans et al, 2000; Sills et al, 1997).

1.5 INTRACEREBRAL MICRODIALYSIS

1.5.1 Introduction

Microdialysis was originally developed as a technique to obtain information about the concentration of endogenous compounds in brain ECF (Fenstermacher et al, 1981). After several decades, the technique of microdialysis has been improved greatly and it is now widely used for investigating drug concentrations, transporters, amino acids and neurotransmitters in different tissues and organs, especially in the brain. (Reddy, 1998; Khan et al, 1999; Potschka et al, 2002 and 2004).

1.5.2 Principle of microdialysis

The microdialysis technique employs dialysis principle, in which a membrane, permeable to water and small solutes, is introduced into the tissue. The key elements in microdialysis are the dialysis probe, connective tubing, perfusion medium, prefusion pump and sample collection device (Figure 1.10). The basic tool for microdialysis sampling is a semipermeable dialysis membrane in a microdialysis probe (Figure 1.11).

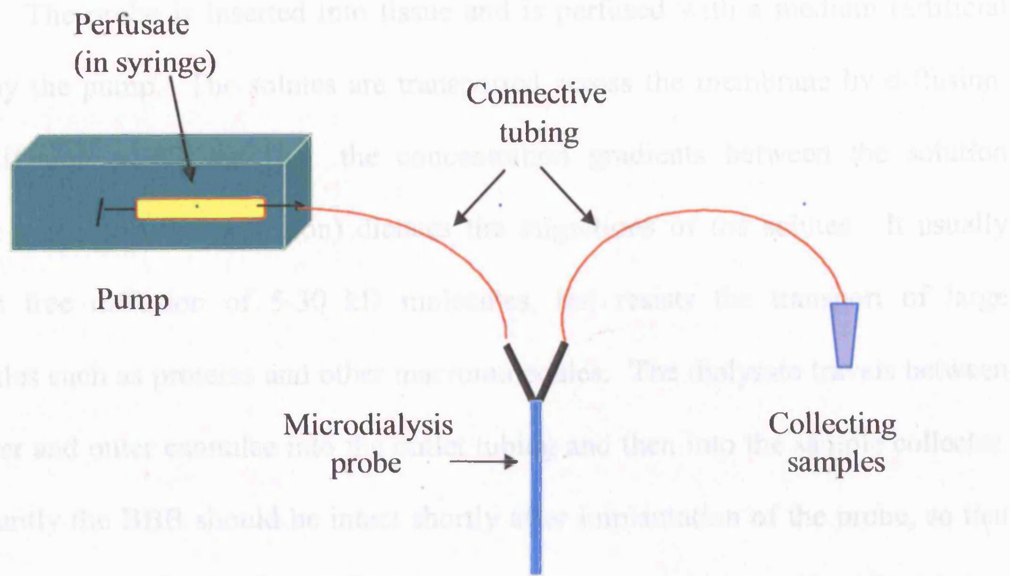


Figure 1.10: Schematic diagram of a basic microdialysis system consisting of syringe pump for perfusate delivery, microdialysis probe, and connective tubing.

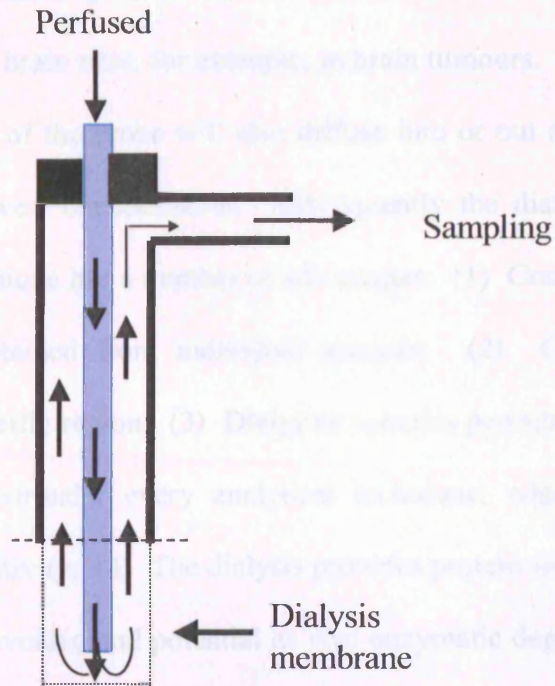


Figure 1.11: Illustration of the microdialysis probe with perfusion tube, sampling tube and dialysis membrane.

The probe is inserted into tissue and is perfused with a medium (artificial CSF) by the pump. The solutes are transported across the membrane by diffusion. The diffusion coefficient (e.g. the concentration gradients between the solution outside probe and the perfusion) dictates the migrations of the solutes. It usually permits free diffusion of 5-30 kD molecules, but resists the transport of large molecules such as proteins and other macromolecules. The dialysate travels between the inner and outer cannulae into the outlet tubing and then into the sample collector. Importantly the BBB should be intact shortly after implantation of the probe, so that samples can be collected from ECF with a continuous perfusion. Therefore, it is a suitable method for studying of amino acids and drug concentrations in the brain tissues of animal models and human.

Pharmacokinetic profiles can be obtained in normal brain as well as in affected or diseased brain sites, for example, in brain tumours. Substances around the semipermeable part of the probe will also diffuse into or out of the perfusate, in the direction of the lowest concentration. Subsequently the dialysate is collected for analysis. This technique has a number of advantages: (1) Concentration versus time profiles can be obtained from individual animals; (2) Concentrations can be determined in a specific region; (3) Dialysate samples permits measurement of drug concentrations by virtually every analytical technique, which contributes to the selectivity and sensitivity; (4) The dialysis provides protein free samples so clean up procedures can be avoided and potential *ex vivo* enzymatic degradation is eliminated; (5) The dialysate samples can be measured for drug and amino acid content at the same time and thus studies of drug mechanisms can be undertaken.

1.5.3 Neuropharmacokinetic studies of new AEDs using microdialysis

In animal studies, the microdialysis technique has been used to investigate the neuropharmacokinetics of a variety of new AEDs including felbamate (Potschka et al, 2002 & 2003), gabapentin (Welty et al, 1993; Wang & Welty, 1996; Rada et al, 1998; Luer et al, 1999; Timmerman et al, 2000), pregabalin (Feng et al, 2001), topiramate (Masucci et al, 1998), lamotrigine (Walker et al, 2000; Potschka et al, 2002 & 2003; Ahmad et al, 2005), levetiracetam (Potschka et al, 2004) and oxcarbazepine (Van Belle et al, 1995; Clinckers et al, 2005).

Potschka et al (2002& 2003) reported that P-Glycoprotein (Pgp, multiple drug transport) and multidrug resistance protein, ABCC2, -mediate efflux of felbamate and lamotrigine at BBB from rat frontal cortex. In these experiments verapamil, a PGP inhibitor, was prefused into the frontal cortex via a microdialysis probe and microdialysate felbamate and lamotrigine concentrations were measured. It was observed that both felbamate and lamotrigine concentrations were increased, and therefore Pgp in brain tissue is likely to limit brain access of felbamate and lamotrigine. This mechanism may play a crucial role in the phenomenon of drug-refractory epilepsy (Potschka et al, 2002). Interestingly, there were no significant differences in felbamate and lamotrigine concentrations in ECF between nonmutant rats and ABCC2-deficient TR rats, which suggest that ABCC2 does not affect modulate felbamate and lamotrigine entry into brain (Potschka et al, 2003). In a further study, Potschka et al (2004) reported that ECF levetiracetam concentrations were unaffected by administration of a variety of multidrug transporter (Pgp or MRP1/2) protein inhibitors.

The concentration versus time profiles of gabapentin in plasma and brain were determined following a single 15mg/kg intravenous bolus dose of rats (Welty et al, 1993). ECF gabapentin concentrations were observed to be only 3-6% of the plasma concentrations and that the anticonvulsant effect of gabapentin was delayed by time-dependent events other than distribution from blood to brain (Welty et al, 1993). Wang and Welty (1996) reported on the influx and efflux BBB permeabilities of gabapentin using a microdialysis pharmacokinetic approach. They observed that the total brain tissue concentration of gabapentin was significantly higher than these in the ECF. Thus it would appear that gabapentin accumulated in intracellular or bounded to brain tissue. Using microdialysis techniques, Luer et al (1999) determined gabapentin concentrations in the brain ECF; these coincided with proportional changes in plasma concentrations. The ratios of AUC_{ECF} to AUC_{plasma} and brain ECF to plasma gabapentin concentration for each collection interval were determined to provide indicators of the relative amount of gabapentin crossing the BBB. Although higher AUC_{ECF} values were obtained with higher AUC_{plasma} values, changes in AUC_{ECF} were less than proportional to observed changes in AUC_{plasma}. BBB saturation of gabapentin transport was evident as the AUC_{ECF} ratio decreased with increased AUC_{plasma} ratio. These result support the carrier-mediated transport mechanism of gabapentin through the BBB.

The rate of brain penetration of lamotrigine and topiramate were also investigated by microdialysis in a rat model (Walker et al, 2000; Masucci et al, 1998). Using *in vivo* recovery corrected microdialysis in the frontal cortex and the hippocampus, the calculated penetration rate of lamotrigine was observed to be not significantly different from that for CSF (Walker et al, 2000). The concentrations of

lamotrigine in ECF were similar to those for CSF, but concentrations of lamotrigine in plasma were much higher than that found in the ECF and CSF. Furthermore, lamotrigine concentrations in the hippocampus were higher than those in the frontal cortex. Using *in vivo* microdialysis, the neuropharmacokinetics of topiramate was studied by Masucci et al (1998) who reported that the C_{max} of topiramate in brain ECF was approximately 10μM and the T_{max} occurred at 45 minutes post topiramate (50mg/kg) i.v. administration.

In human neuropharmacokinetic studies, Alves et al (2003) used intracerebral microdialysis to provide a safe and efficient tool for continuous *in vivo* evaluation of the bioavailability and efficacy of topiramate. Topiramate crossed the BBB at concentrations that may be neuroprotective, and could modulate glutamate concentrations. In a single patient with drug-resistant partial epilepsy study (Lindberger et al, 1999), topiramate concentrations were measured in three different compartments (brain ECF, CSF and plasma). The mean of ECF/CSF topiramate ratio was 0.93 and protein bounding of topiramate in plasma was approximately 13%. Topiramate concentrations in the CSF were approximately the same as the unbound plasma concentrations.

At present, microdialysis is becoming an increasingly useful tool for monitoring AED concentrations in the brains of patient that are undergoing investigation for possible brain surgery for the treatment of their epilepsy (Lindberger et al, 1998 & 2001; Hammarlund-Udenaes, 2000).

1.5.4 Amino acid studies of new AEDs using microdialysis

As highlighted earlier, microdialysis can assess mechanisms and pharmacodynamic actions of AEDs. Most studies in this respect were undertaken in animal models and relate to AEDs that have GABAergic mechanisms. Jolkkonen et al (1992) first investigated the acute effect of vigabatrin on the GABAergic system in a rat model by using microdialysis. They perfused vigabatrin via the microdialysis probe and found the immediate increase of GABA outflow, which suggested that the result may be due to the direct blockade of GABA uptake sites and concluded that GABA-T inhibitors may be mediated partly through GABA uptake inhibition. In the limbic seizure model, GABA, glutamate and dopamine concentrations in the hippocampus were studied. 5 mM vigabatrin was perfused into rat hippocampus via a microdialysis probe and it was observed that GABA concentrations were increased and glutamate and dopamine concentrations were decreased (Smolders et al, 1997).

The effect of topiramate on excitatory amino acids were investigated using *in vivo* microdialysis in hippocampus (Kanda et al, 1996). Topiramate (40mg/kg I.P.) reduced both glutamate and aspartate concentrations in the hippocampus of SER. Lamotrigine and oxcarbazepine also inhibited extracellular glutamate and aspartate levels in different animal models (Waldmeier et al, 1996; Bacher & Zornow, 1997). The results showed that lamotrigine and oxcarbazepine were effective in inhibiting extracellular glutamate which might explain the neuroprotective properties of lamotrigine and oxcarbazepine.

To gain insight into the mechanism of action of the AEDs gabapentin, the effects of gabapentin on the *in vivo* ECF GABA concentration in rat brain were

studied using microdialysis. Gabapentin was studied under basal, K+, nipecotic acid and glutamate-stimulated conditions. Gabapentin administered at a dose of 100mg/kg that did not have any significantly effect on ECF GABA concentrations, regardless of experimental conditions (Timmerman et al, 2000). A study was also carried on the effect of gabapentin on spinal cord amino acid release (Feng et al, 2003). Gabapentin was administered at various doses (50, 100, or 200 mg/kg) and dialysate was collected from the spinal subarachnoid space. It was observed that gabapentin effectively inhibited the acetic acid evoked release of excitatory amino acids in the spinal cord.

The effects of combined lamotrigine and valproate on ECF amino acids with different drug concentrations were investigated in rat microdialysis (Ahmad et al, 2005). Lamotrigine (10mg/kg) and valproate (300mg/kg) did not significantly alter basal levels of aspirate, glutamate or taurine concentration when they were given alone. However, the two drug administered together significantly reduced ECF aspartate and glutamate concentrations while increasing taurine levels. Additionally, only valproate increased ECF GABA concentrations. Pharmacokinetic profiles in three compartments were unchanged after co-administration of lamotrigine and valproate. These results suggest that the changes of the amino acids are unlikely due to pharmacokinetic contribution and probably pharmacodynamic interaction of the two drugs.

1.6 PAIRED-PULSE INHIBITION

1.6.1 Introduction

Paired-pulse stimulation is well-suited to detect excitability changes in hippocampal pathways, since the first or 'conditioning' stimulus acts to recruit recurrent excitatory (Cronin et al, 1992) or inhibitory activity (Sloviter, 1991) in these pathways.

1.6.2 The mechanisms of paired pulse inhibition and hippocampus

One of the most frequently tested sites for paired-pulse inhibition is the dentate gyrus of the hippocampus. In the hippocampus, neuronal population can be divided into two broad classes: principal neurones that are excitatory and use glutamate as a neurotransmitter and inter-neurones which are inhibitory and use GABA as a neurotransmitter (Freund & Buzsaki, 1996). In normal tissue, principal neurones, which represent 90% of the neuronal population, are under the control of inter-neurones. Alteration of the balance of inhibitory and excitatory is one of the hypotheses for mechanisms underlying the occurrence of epilepsy. Paired-pulse inhibition reflects inhibition in the hippocampus

In the paired-pulse protocol, the first pulse delivered to the input pathway, activates interneurons (feed forward inhibitions) and firing of the target neurons can also recruit interneurons (feed back inhibition). The second pulse will then be inhibited if the inter-pulse interval (IPI) falls within the period of recurrent or feed-forward and feedback inhibitions (Figure 1.12).

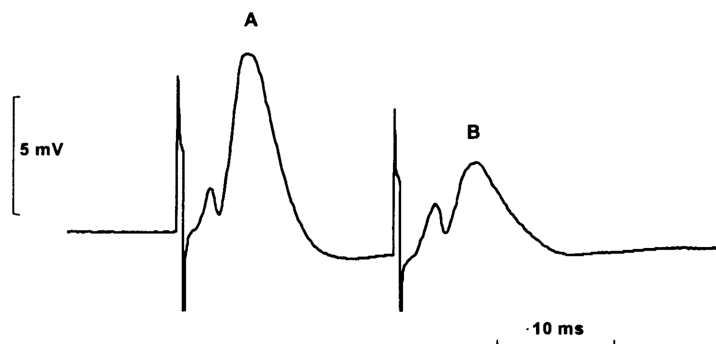


Figure 1.12: Paired-pulse inhibition in the perforant path model of status epilepticus. The pulse-interval was 20 ms. A, the first pulse, delivered to the input pathway, activates cell discharge. B, the second pulse, is inhibited via the feed-forward and feed-back inhibition.

Hippocampal inhibition has been studied extensively using field potential recordings and paired-pulse stimulation, and is measured by the inhibition in the second (test) population spike (PS) and excitatory postsynaptic potential (EPSP) slope. This inhibition is mediated over short interpulse interval by $GABA_A$ receptors and by $GABA_B$ receptors over longer time intervals. (Figure 1.13).

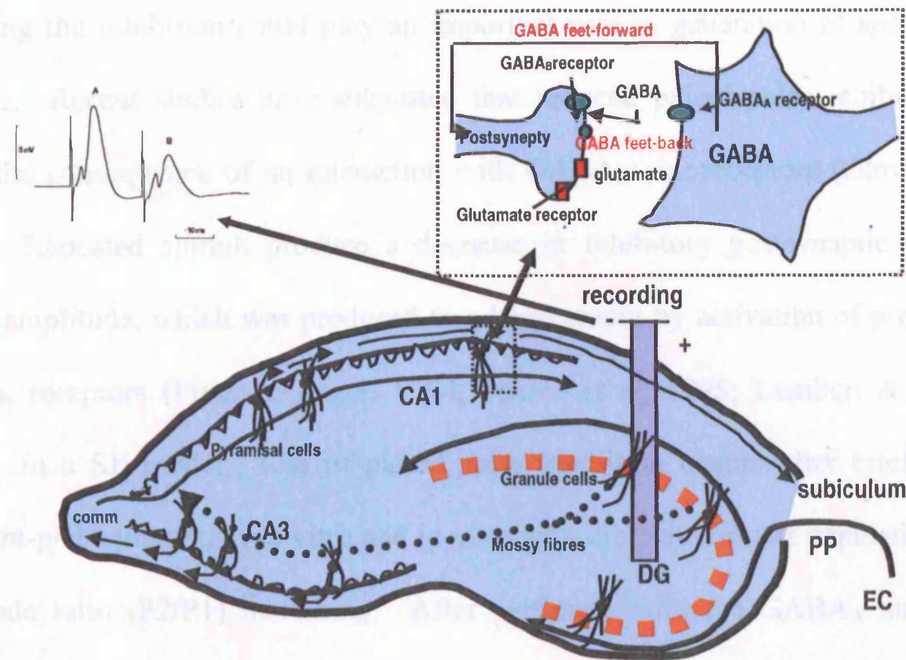


Figure 1.13: Schematic diagram of mechanism of paired-pulse inhibition in hippocampus.

1.6.3 Paired-pulse inhibition in epilepsy

Several studies have used the paired-pulse technique to monitor inhibition in the dentate gyrus during epilepsy in animal models (Sloviter, 1987; Sayin et al, 2003; Kobayashi & Buckmaster, 2003, Naylor & Wasterlain, 2005). An early study (Sloviter,1987) reported a decrease in paired-pulse inhibition in the dentate gyrus following induction of SE by prolonged periods of electrical stimulation. Similar results were also found by Haas and his colleagues (1996). Sayin et al (2003) reported that paired-pulse inhibition in the dentate gyrus was reduced or lost after approximately 90-100 evoked seizures in association with emergence of spontaneous seizures. The loss of paired-pulse inhibition was accompanied by loss of subclasses of inhibitory inter-neurones labelled by cholecystokinin and the neuronal GABA transport GAT-1. These indicated that seizure-induced loss of inter-neurones

providing the inhibition could play an important role in generation of spontaneous seizures. Recent studies have suggested that reduced paired-pulse inhibition was likely the consequence of an interaction with $GABA_B$ autoreceptors (Gloveli et al, 2003). Repeated stimuli produce a decrease in inhibitory postsynaptic potential (IPSP) amplitude, which was produced to a large extent by activation of presynaptic $GABA_B$ receptors (Pitler & Alger, 1994; Pearce et al, 1995; Lambert & Wilson, 1993). In a SE model, loss of paired-pulse inhibition occurs after brief 5 mins perforant-path stimulation in vitro and in vivo with the paired-pulse population spike amplitude ratio (P2/P1) increasing. After perfusion with the $GABA_A$ antagonist, bicuculline, the ratio also increased from a baseline. These results suggest that loss of inhibition at GABA synapses may be an important early event in the initiation of SE (Naylor & Wasterlain, 2005).

Most studies of paired-pulse inhibition in the human are using hippocampal slices from temporal lobe epileptic patients (Urun et al, 1994 & 1995; Swanson et al, 1998) and only a few *in vivo* studies in human have been performed (Wilson et al, 1998). Uruno et al (1995) examined brain slices from temporal lobe epileptic patients. Paired-pulse depression (PPD) was examined using perforant path stimulation of low to moderated intensity at an inter-stimulus interval of 20ms. The results are consistent with less or reduction of feedback inhibition by bicuculline and baclofen, which occurred paired-pulse facilitation in the dentate gyrus of temporal lobe epileptic patients. The data suggests the re-evaluation of the role of inhibition in epilepsy. Swanson et al (1998) also analyzed paired-pulse depression in dentate slices from 9 patients with temporal lobe epilepsy. Their data showed that comparing feedback inhibition between human and rat tissue, the human inhibition was much

greater, increased in amplitude and occurring at all inter-stimulus intervals. This increased feedback inhibition could reflect species differences between rat and human dentate physiology, or be a unique feature of epileptic dentate. Wilson et al (1998) used paired-pulse stimulation to investigate changes in excitability in anaesthetized patients with temporal lobe epilepsy. A total of 20 patients with temporal lobe epilepsy were studied during chronic depth electrode monitoring for seizure localization. The mean paired-pulse inhibition was significantly greater for epileptogenic perforant path response than for contralateral perforant path responses. The results suggest that enhanced inhibition may be homeostatic mechanism to inhibit seizures and maintain the interictal state, ultimately contributing to the characteristic of the epileptogenic hippocampus.

1.6.4 Paired-pulse inhibition and new AEDs.

Paired-pulse stimulation is a technique that is largely used to assess convulsant and anticonvulsat drugs for GABA inhibition (Stringer, 2000; Stringer & Taylor, 2000). In animal models, Xiong and Stringer (1997) investigated the effects of three new AEDs, felbamate, lamotrigine and gabapentin on excitability and inhibition in rat hippocampus. They found that all three drugs caused a dose-dependent decrease in the duration of seizure discharge. None of the drugs altered excitability, while only gabapentin altered (decreased) paired-pulse inhibition. The effect of gabapentin on paired-pulse inhibition suggested a proconvulsant rather than antiepileptic effect. The same result of gabapentin reduced paired-pulse inhibition was also found in urethane-anesthetized model (String & Lorenzo, 1999). Pre-treatment with vigabatrin, had no effect on the ability of gabapentin to reduce paired-

pulse inhibition. These data suggest that the mechanism of action of gabapentin is probably not through an increase in basal levels of GABA (String & Aribi, 2002). Topiramate has several anticonvulsant mechanisms. Both in animal model and in hippocampus slices demonstrated that topiramate was associated with an increase in paired-pulse inhibition (Wu et al, 1998; String, 2000).

The acute of effects of vigabatrin on GABAergic inhibition were investigated in the hippocampal slice using paired-pulse inhibition. The results showed that a concentration-dependent decrease in GABAergic inhibition (Jackson et al, 1994a). Similar results were also seen *in vitro* and *in vivo* studies (Sayin et al, 2001). The effect of vigabatrin on paired-pulse inhibition in the rat dentate gryrus was investigated by perforant path stimulation. After administration 1g/kg vigabatrin (I.P.), there was a loss paired-pulse inhibition at both 15 and 25 ms interpulse intervals (IPI). Sayin et al (2001) suggested that activation of presynaptic $GABA_B$ receptors, by increased extracellular GABA, might be one of the contributing factors to the apparent paradoxical effect of vigabatrin on paired-pulse inhibition.

As paired-pulse inhibition is an accepted technique to functionally assess GABAergic mechanisms, Margineanu and Wulfert (1995) investigated the effects of paired-pulse inhibition of levetiracetam in the bicuculline seizure model. The results showed that levetiracetam neither affected paired-pulse inhibition nor reduced paired-pulse inhibition in bicuculline-induced hyperexcitability. Subsequently Margineanu and Klitgaard (2003) investigated whether levetiracetam affected the paired-pulse inhibition of the field potentials evoked in the dentate gyrus of urethane-anesthesized rats. This model revealed a strong paired-pulse inhibition at 20ms interstimulus

interval. Bicuculline and baclofen markedly depressed paired-pulse inhibition at 20ms interstimulus interval, whiles clonazepam, diazepam and phenobarbital enhanced paired-pulse inhibition. In contrast to these $GABA_A$ and $GABA_B$ receptors acting drugs, levetiracetam has no significant effect on paired-pulse inhibition interaction in the rat; this would suggest that the mechanism of action of levetiracetam does not involve in the GABAergic mechanisms (Margineanu & Klitgaard, 2003).

1.7 AIMS OF THE STUDY

Using an animal model that allows the temporal (blood) pharmacokinetics and central (CSF and ECF) brain neuropharmacokinetics of a drug and pharmacodynamic correlates (amino acid neurotransmitters), and an electrophysiological model, this thesis sought to investigate various aspect of two new AEDs (vigabatrin and levetiracetam).

1. The blood pharmacokinetics and CSF and ECF neuropharmacokinetics of vigabatrin after acute intraperitoneal administration were compared.

2. The blood pharmacokinetics and ECF neuropharmacokinetics of levetiracetam after intraperitoneal administration were determined.

3. The effect of vigabatrin and levetiracetam on candidate amino acid neurotransmitters in CSF and ECF were determined and compared.

4. The effects of vigabatrin and levetiracetam in paired-pulse inhibition were determined and compared.

Chapter 2

Methodology

2.1 MATERIALS

2.1.1 Animals

Male Sprague-Dawley rats (Charles River, Margate, Kent, U.K.), weighing 300-350 g, were used in all experiments. Rats were individually housed in groups of 3 for 5-7 days prior to surgery and were allowed free access to water and to a normal laboratory diet (SDS R and M number 1 expanded, Scientific Dietary Services, Witham, Essex, U.K.). A 12 hours light/dark cycle (light on 07:00 hours) and an ambient temperature of 25°C were maintained.

2.1.2 Antiepileptic drugs

Vigabatrin was obtained from Hoechst Marion Roussel Ltd (Kingfisher Driver, Swindon. U.K.). Levetiracetam was supplied by the UCB Pharmaceutical Sector (Chemin du Fories, Belgium). Since these two AEDs are hydrophilic and water soluble, saline was used as the vehicle of choice.

2.1.3 Blood catheter and CSF cannulae

The blood catheter comprised of a 5 cm silastic tubing (2.5mm ID and 1.1mm OD, Bio pure technology Ltd, UK) connected to a 12 cm polythene tubing (0.58 mm ID and 0.96mm OD, SIMS portex Ltd, UK). Catheters were constructed by connecting these two tubings together by inserting the polythene tubing into the silastic tubing (Figure 2.1). The silastic tubing was fixed in the jugular vein whilst the polythene tubing was used for collecting blood samples and accessible externally (Figure 2.1).

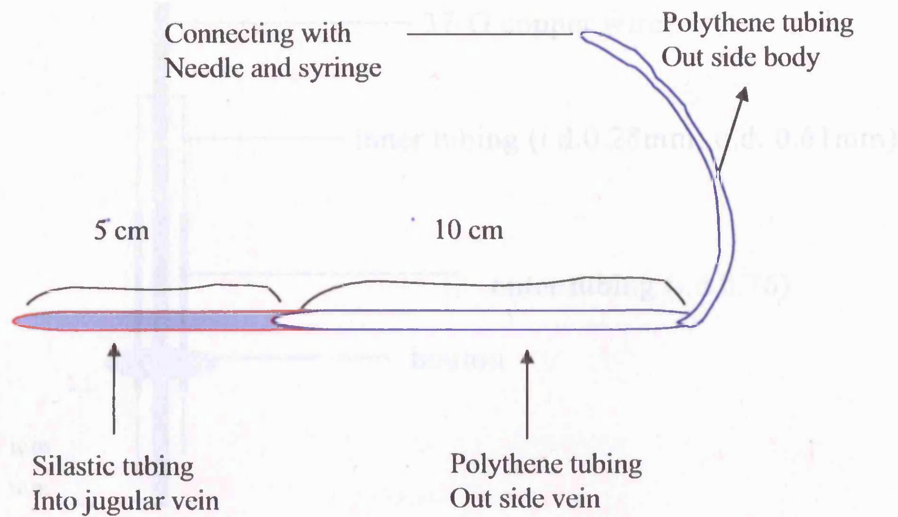


Figure 2.1: A schematic of a blood catheter.

The construction of the CSF cannulae was as described by Patsalos et al (1992). The CSF cannula comprised of an outer (0.58 mm ID and 0.96mm OD) and inner (0.26 mm ID and 0.61mm OD) polythene tubings (Figure 2.2). The dimensions of both tubing were specifically chosen to allow insertion (with tweezers) of the inner polythene tubing into the outer polythene tubing after the button was formed, but not to allow the inner tubing to be readily withdrawn. The button was formed by gentle heating of the catheter over a soldering iron. This resulted in a tight fit around the inner polythene tubing (0.28mm ID, 0.61mm OD). A 37 G copper wire (Scientific Wire Co., London, UK) was inserted through the inner tubing. This afforded rigidity to the catheter and aided the puncturing of the brain dura.

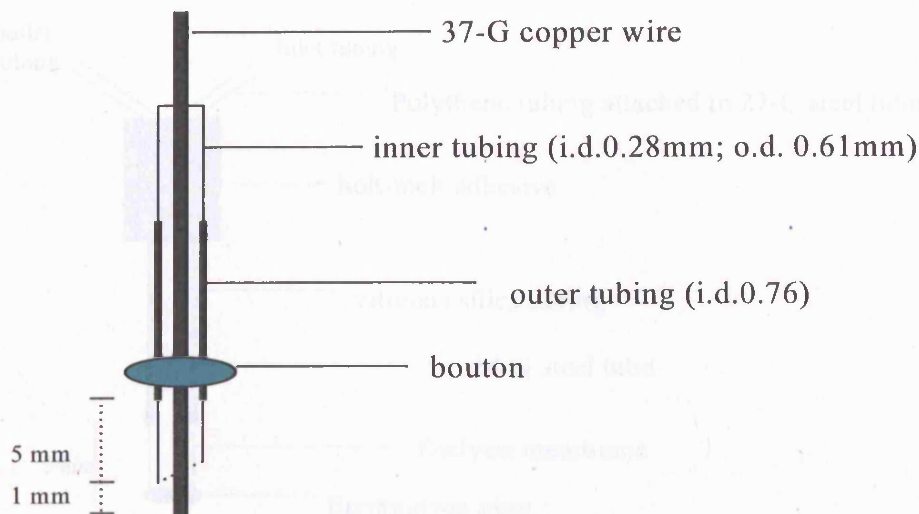


Figure 2.2: A schematic of a CSF cannula.

2.1.4 Microdialysis probe

Concentric microdialysis probes with filtral 12 and a dialysis membrane (4 mm long, 200 μM diameter, Hospal Rugby, U.K.) were used. The probes were constructed as Figure 2.3 and internal tubes were used by vitreous silica tubing (SGE, Milton Keynes, UK). Two silica tubes were held in place in a 15 mm 24-G steel tube with epoxy resin. The dialysis membrane was placed over the 4 mm protruding silica tube and passed up into the steel tubing. The membrane was then fixed in place and the end with epoxy resin. Two 27-G steel tubes (8 mm long) were inserted into the polythene tubing (i.d. 0.28 mm), placed over the silica tubing at the other end and held in place with epoxy resin. These were then sealed with hot melted adhesive.

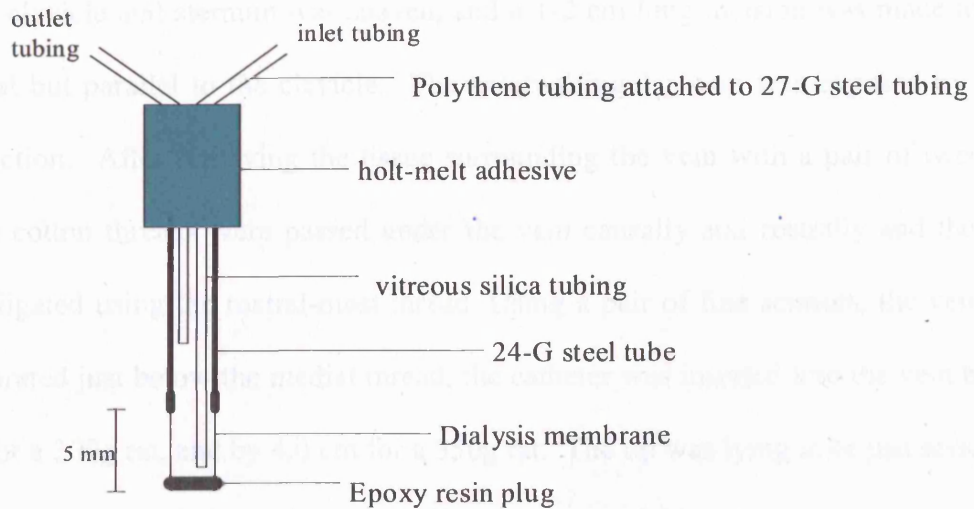


Figure 2.3: A schematic of a microdialysis probe.

2.2 SURGICAL PROCEDURES AND SAMPLING

2.2.1 Anaesthetics

All surgical procedures were performed under general anaesthesia according to Home Office ANIMALS (SCIENTIFIC PROCEDURES) ACT 1986. The rats were first placed in an induction chamber with 4 % halothane (May & Baker Ltd, Dagenham, UK) in oxygen for about 10 min. When rats became unresponsive to foot pinch, they were transferred to a stereotaxic frame for surgery (Stoelting Co. IL, USA). Halothane (1-2%) in oxygen at a flow rate of 2L/min was provided via a specially constructed facemask until the surgery was completed.

2.2.2 Surgery for blood catheter and CSF cannulae insertion

The procedure for inserting the blood catheter and CSF cannula was as previously described (Patsalos et al, 1992). Rats were firstly placed on their backs, and fixed by their forelegs to a surgical table by adhesive tape. The skin between the

right clavicle and sternum was shaved, and a 1-2 cm long incision was made in skin rostral but parallel to the clavicle. The external jugular vein was exposed by blunt dissection. After removing the tissue surrounding the vein with a pair of tweezers, three cotton threads were passed under the vein causally and rostrally and the vein was ligated using the rostral-most thread. Using a pair of fine scissors, the vein was perforated just below the medial thread, the catheter was inserted into the vein by 3.5 cm for a 300g rat, and by 4.0 cm for a 350g rat. The tip was lying in or just above the right ventricle, and blood flowed readily in and out of the catheter. The caudal-most thread was tied around the vein thus holding the catheter within the vein, the other two threads were tied around the catheter to keep it in place. A cut was then made just behind the right ear and the catheter was passed under the skin and through this incision. The wounds were then sutured. The catheter was further flushed with 5U/l heparin and the end was cut so that 2-3 cm protruded out of the skin, and the end of the catheter was tied.

For the CSF cannulae surgery, the animal was placed in a stereotaxic frame with both ear bars set at zero and the nose piece set to + 5 mm. The skin and the atlantoccipital membrane were retracted. Two burr holes were made, the first was 3 mm lateral and 4 mm caudal to lambda for the placement of a stainless steel anchor screw, and the second was 2 mm lateral to the midline and 3 mm caudal to the external occipital crest and at an angle of about 40° for the catheter insertion. The cannulae were inserted until the button rested on the skull surface. The wire was then withdrawn and the cannulae sealed in place with dental acrylic (De Trey, Weybridge, Surrey, UK). CSF was introduced into the cannulae by a slight negative pressure using a syringe. Finally, the cannula was cut to approximately 2 cm, and heat sealed.

After surgery, rats were housed individually in perspex cages and allowed food and water ad libitum.

2.2.3 Surgery for the microdialysis probe insertion

Rats were anaesthetised as described previously. The hair on the head was clipped and the skin surface was sterilised using an alcoholic sterile. The animal was then placed in a stereotaxic frame with both ear bars set at zero and the nose piece set to + 5 mm. A 2 cm incision was made 8 mm caudal to lambda and external occipital crest. A small piece of skin was excised (about 0.5 cm wide and 1.0 cm long), and the atlantoccipital membrane was retracted. Microdialysate probes were implanted in either the hippocampus (from bregma 5.3 mm posterior, 4.5 mm lateral, 7.5 mm ventral), or the frontal cortex (from bregma 3.0 mm anterior, 2.5 mm lateral, and 5.0 mm ventral) according to the atlas of Paxinos and Watson (1986). Two burr holes were made for two screws fixing the probe. The probes were sealed in place with dental acrylic cement (De Trey, Wey-bridge, Surrey, UK).

2.2.4 Blood and CSF sampling

Blood sampling was achieved by connecting the jugular vein catheter via a 1.5 cm stainless steel connector to a 60 cm polythene tubing (i.d. 0.58 mm). Blood was slowly withdrawn until it reached the bottom of the syringe containing the 10U/ml heparin. The cannula was closed using a small clip and a new syringe was attached. 0.1 ml of blood was withdrawn to the syringe. In order to prevent the development of hypovolaemia, an equivalent volume of heparinized saline was administered after each sampling. Two baseline samples were taken at 30 min intervals before drug administration, then blood samples were withdrawn at 20 min

intervals in the first hour, and at 30 min intervals for the subsequent 7 hours. Blood samples were collected into 0.5 mL centrifuge tubes and then centrifuged for 5 min at 11,000 g to separate the cells from the serum. The serum was transferred into a new tube and stored at -70°C until required for analysis.

Sampling of the CSF was achieved by connecting the implanted cannulae to a tube attached to a 23 G needle and 1 mL syringe. The tube consisted of 30 cm (i.d. 0.28 cm) polythene tubing, which was attached to 40 cm (i.d. 0.58 mm) polythene tubing. The polythene tubing (i.d. 0.58 mm) was marked at 8 cm with a marker pen, and this corresponded to a volume of 20 μL. Two baseline samples were taken at 30 min intervals before drug administration, and CSF samples withdrawn at 30 min interval or up to 8 hours. CSF was dispensed into 0.5 mL polypropylene tubes and stored at –70°C until required for analysis.

2.2.5. Microdialysate (ECF) sampling

The ECF sampling was achieved by connecting the implanted microdialysis probe with 20 cm polythene tubing (i.d. 0.28 mm). During the first 2 hours, the dialysate sample was discarded so as to allow stable sampling conditions to be established. Then base line samples were collected by an hour and 15 minutes for each sample point. After drug administration, dialysate samples were collected at 20 min intervals for 4 hours and then for 30 min intervals for a further 4 hours. Dialysate samples were collected into 0.5 mL polythene tubes (Treff AG, Switzerland) and stored at -70°C until they were analysed for drug and amino acid content.

2.2.6 Microdialysis pump, artificial CSF and microdialysis probe recovery

A Harvard Apparatus 22 micro-pump (Model: 55-22228, Harvard, U.S.A.) was used. The microdialysis flow rate was typically 1-2 μL/min but was varied for different experiments. A 1 mL syringe was fixed to the pump containing artificial CSF (composition in mM: NaCl 125, KCl 2.5, $MgCl_2$ 1.18 and $CaCl_2$ 1.26).

In vitro recovery for each probe was determined by placing the probe in a beaker containing a 40 μmol/L drug solution constituted in artificial CSF. The probes were then perfused at 1 μL/min for vigabatrin and 2 μL/min for levetiracetam. Samples were collected every 20 min for 1 hour and stored at -70°C until analysis for drug content.

2.3 SAMPLE ANALYSIS

The analyses of vigabatrin, levetiracetam and amino acids were undertaken by high performance liquid chromatography (HPLC) and were based on the methods described by Ratnaraj & Patsalos (1998) and Ratnaraj et al (1996).

2.3.1 Measurement of vigabatrin concentration

Vigabatrin serum concentration (total and free non-protein bound) were determined using HPLC with fluorescene detection as previously described (Ratanraj & Patsalos, 1998). All solvents were of HPLC grade and reagents were of "Analar" grade, purchased from BDH-Merck (Poole, UK).

2.3.1.1 Extraction procedure

Vigabatrin serum and CSF concentrations were measured by adding 30 µL serum, 15 µL CSF or ECF into 1.5 mL polypropylene microcentrifuge tube (Scltkab Ltd, Coat-bridge, Lanarkshire, UK), and adding ortho-phthaldialdehyde (OPA) 60 µL for serum and 30 µL for CSF and ECF respectively. Samples were then mixed for 10 seconds using a Vibrax electronic shaker (Sartorius-IKA, Epsom, U.K.) and centrifuged for 5 min at 11,000 g using a microcentrifuge (Abbott, Maidenhead, U.K.). The supernatant (80 µL for serum and 40 µL for CSF and ECF) was transferred into a Chromacol vial (Anachen Ltd, Bedfordshire, U.K.) and the vial sealed with a screw cap. The vials were then loaded into the HPLC autosampler. 10 µL samples were subsequently injected into the chromatograph.

2.3.1.2 HPLC instrumentation and chromatographic condition

A Gilson HPLC (Anachem Ltd, Luton, UK) was used and comprised of the following modules: a Gilson 302 and 305 pump, an autosampler (Gilson model 234) and a Gilson 811C dynamic mixer. A Perkin Elmer fluorescence detector (Perkin Elmer Ltd, Beaconsfield, Bucks, UK) was used and set at 250 nm and 550 nm. Chromatograms were run at 35°C using a model 7955 column chiller (Jones Chromatography Ltd, Hengoed, Mid. Glamorgan, UK) and a Hypersil BDS-C18, 3 µM, 125x 3 mm column (Hewlett Packard, Stockport, Cheshire) with pre-column LiChrospher select B 4x4 (5µM) (Hewlett Packard, Stockport, Cheshire) was used. Phosphate buffer (145mM) was for line A (Gilson pump 305) and a mobile phase of acetonitrile and water was for line B (Gilson pump 302). Line A and B buffer 42:58 vol/vol was used in the beginning and continually changed to 00 (line A):100 (line B)

vol/vol at a flow rate of 0.45 ml/min and at 2.5 psi. A typical chromatogram for the analyse of vigabatrin is shown in Figure 2.4.

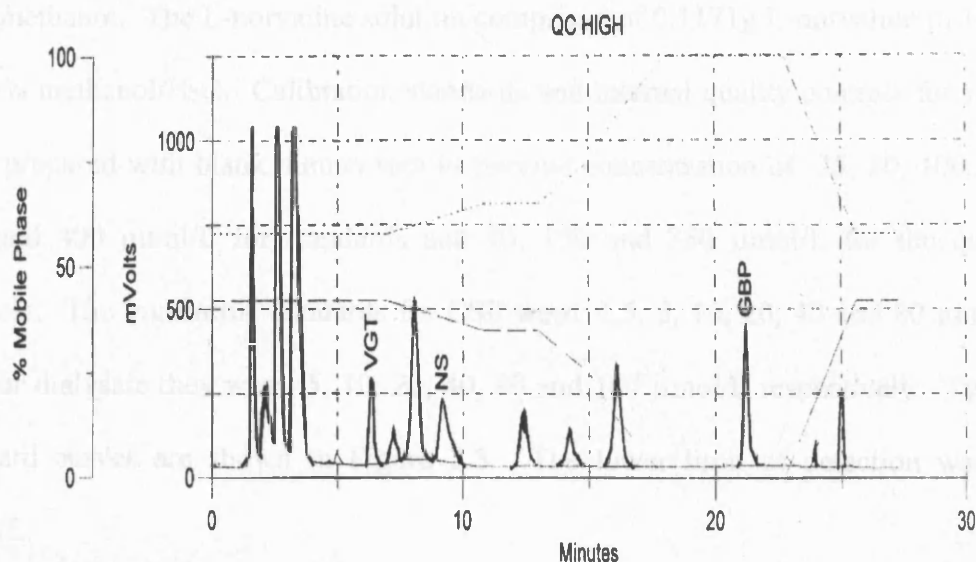


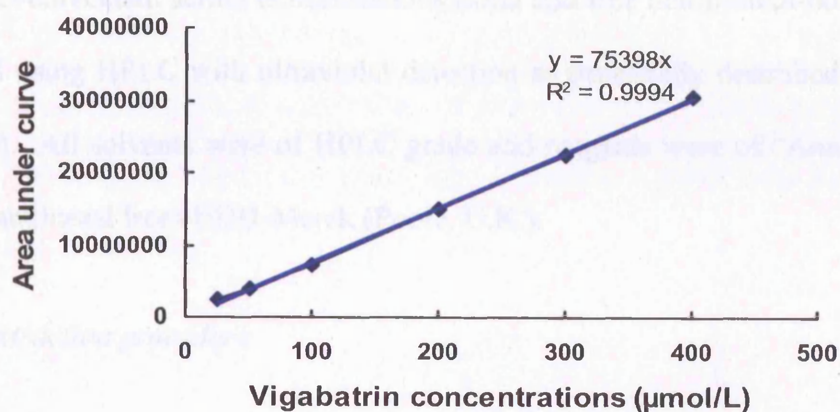
Figure 2.4: A typical HPLC chromatogram of vigabatrin (VGT).

2.3.1.3 *Preparation of buffer and standard solutions*

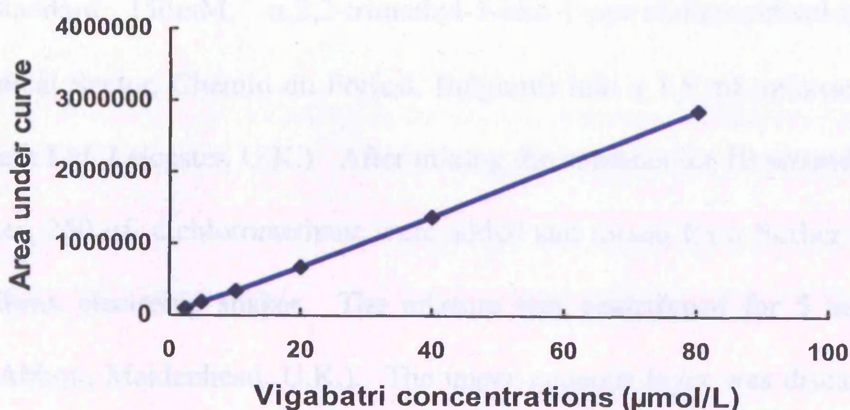
Phosphate buffer (145mM) was prepared by mixing 320 mL of stock solution (phosphate buffer 250 mM), with 95 mL of acetonitrile and 550 mL water. A final pH of 6.5 was obtained by using 5M sodium hydroxide (NaOH). This was passed through line A of the HPLC. Line B comprised of a mobile phase of 280 mL acetonitrile, 240 mL methyl alcohol (MeOH) and 480 mL water. The borate buffer (0.4M) was made of boric acid in water and adjusted to pH 10.5 using 5 M NaOH. The OPA (Sigma, Poole, Essex, BDH, Dagenham, Essex, UK) derivitising reagent consisted of 0.4 mL MeOH, 3.6 mL borate buffer and 50 μL 2-mercaptoethanol (Sigma, UK).

Area under the peak corresponding to vigabatrin was calculated and was compared to a standard curve. The internal standard was prepared using L-norvaline with methanol. The L-norvaline solution comprised of 0.1171g L-norvaline in 100ml of 50% methanol/H_2O. Calibration standards and internal quality controls for serum were prepared with blank human sera to provide concentration of 25, 50, 100, 200, 300 and 400 µmol/L for standards and 40, 150 and 350 µmol/L for the quality controls. The vigabatrin standards for CSF were: 2.5, 5, 10, 20, 40 and 80 µmol/L, and for dialysate they were: 5, 10, 20, 40, 80 and 160 µmol/L respectively. Typical standard curves are shown in Figure 2.5. The lower limit of detection was 0.2 µmol/L.

A



B



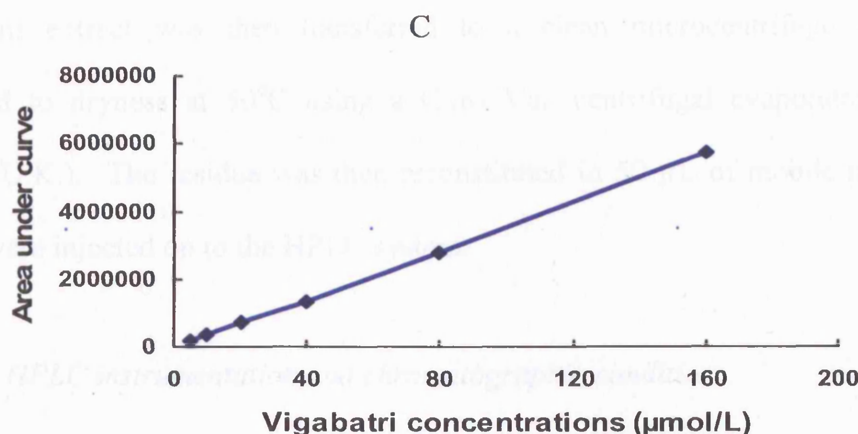


Figure 2.5: Typical vigabatrin standard curves for (A) serum, (B) CSF and (C) dialysate. The least mean squares regression lines are shown.

2.3.2 Measurement of levetiracem concentration

Levetiracetam serum concentrations (total and free non-protein-bound) were determined using HPLC with ultraviolet detection as previously described (Ratanraj et al, 1996). All solvents were of HPLC grade and reagents were of "Analar" grade and were purchased from BDH-Merck (Poole, U.K.).

2.3.2.1 *Extraction procedure*

Letiracetam serum concentrations were measured by adding 15 µL serum and dialysate, 25 µL 5M sodium hydroxide solution and 25 µL of the working internal standard (50mM, α,2,2-trimethyl-5-oxo-1-pyrrolidineacetamide; UCB Pharmaceutical Sector, Chemin du Foriest, Belgium) into a 1.5 mL microcentrifuge tube (Sarstedt Ltd, Leicester, U.K.) After mixing the contents for 10 seconds using a vortex mixer, 250 µL dichloromethane were added and mixed for a further 1 minute using a Vibrax electronic shaker. The mixture was centrifuged for 5 minutes at 11,000 g (Abbott, Maidenhead, U.K.). The upper aqueous layer was discarded and

the solvent extract was then transferred to a clean microcentrifuge tube and evaporated to dryness at 50°C using a Gyro Vap centrifugal evaporator (Howe, Banbury, U.K.). The residue was then reconstituted in 50 µL of mobile phase and samples were injected on to the HPLC system.

2.3.2.2. HPLC instrumentation and chromatographic condition

The HPLC system comprised of a Spectra-Physics spectra system pump P4000, an AS 3000 autosampler, a UV2000 detector and a chromjet integrator. Chromatograms were run at 35°C on a steel cartridge column (250 x 4mm I.D.) with a precolumn (4 x 4 mm I.D.) packed with LiChrospher 60 RP-Select B, 5 µm (Merck, Poole, U.K.). A mobile phase of acetronitrile : 50 mM phosphate buffer pH 5.6 (15:85) with a flow rate of 0.8 mL/min at 1300 psi was used (Figure 2.6 graph from HPLC). The column eluent was monitored at 220 nm with a sensitivity range of 1.0 a.u.f.s and a chart speed of 0.25 cm/min. Figure 2.6 shows a typical chromatogram of levetiracetam.

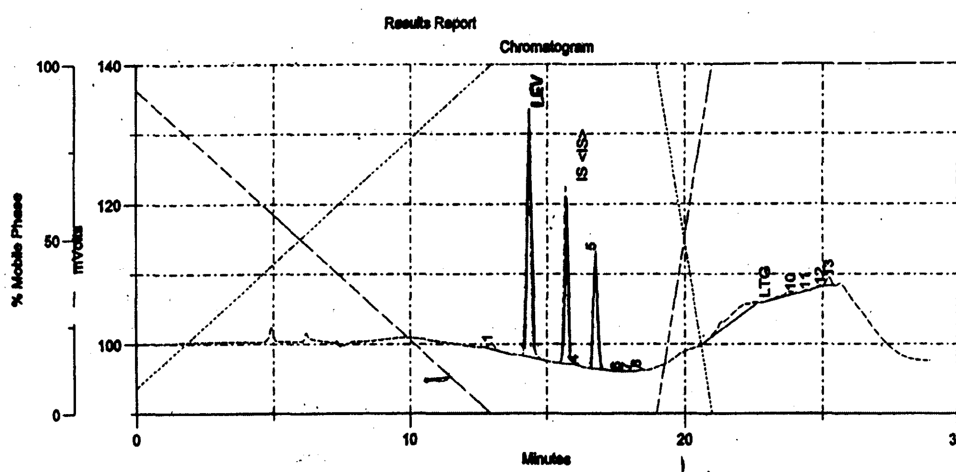


Figure 2.6: A typical HPLC chromatogram of levetiracetam (LEV).

2.3.2.3 Preparations of buffer and standard solutions

Phosphate buffer (50 mM) was prepared by mixing potassium dihydrogen phosphate and water and this comprised buffer A. A second phosphate buffer (50 mM) was prepared from disodium hydrogen phosphate solution and water and this comprised buffer B. 95% of buffer A and 5% of buffer B was mixed together and the pH adjusted to 5.6 by using orthophosphoric acid. A flow rate 0.8 mL/min at 1300 psi was used.

The stock solution of levetiracetam (10 mM) was prepared by using water. The stock solution of ucb 17025, the internal standard (1mg/mL), was prepared using methanol. The internal standard was prepared in 50% acetonitrile and a phosphate buffer (50mM, pH 5.6) mixture to give a final concentration of 217 μmol/L. The levetiracetam standards for serum and dialysate were 25, 50, 100, 200, 300 μM and 2.5, 5, 10, 20, 40, 80 and 160 μM respectively. The quality controls were 40, 75 and 150μmol/L and 8.5 and 26 μmol/L for serum and dialysate respectively. The area under the peak corresponding to levetiracetam was calculated for serum and ECF and expressed as a ratio compared to the area under the peak for the internal standard and these were compared to a standard curve The typical standard curves for serum and dialysate are presented in figure 2.7.

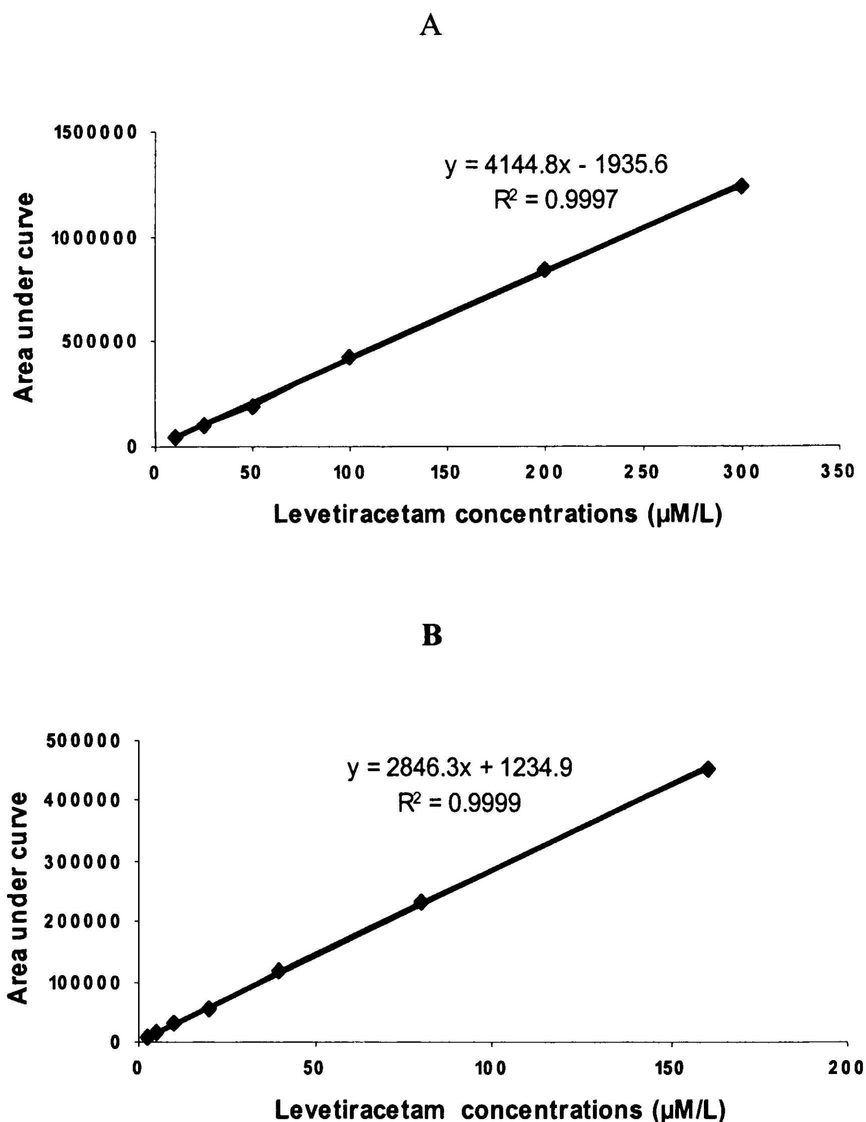


Figure 2.7: Typical levetiracetam standard curves for (A) serum and (B) dialysate. The least mean squares regression lines are shown.

2.3.3 Measurement of free non-protein bound serum vigabatrin and levetiracetam concentration

The procedure used for the determination of the free non-protein bound serum vigabatrin and levetiracetam concentrations was essentially the same as that for total concentration except that samples were first filtered through an Amicon Centrifree Micropartition Systerm (Amicon, Stonehouse, U.K.) by using a Sorvall

RC-5B refrigerated centrifuge (Du Pont, Stevenage, U.K.) set at 25°C. Fifteen μL samples of the resultant ultrafiltrate were then processed as described above.

2.3.4 Measurement of CSF amino acid concentrations

2.3.4.1 HPLC instrumentation and chromatographic condition

The HPLC comprised of Gilson 302 and a 305 pump, a Gilson 234 autosampler, and Unipoint software (Anachem, Luton, UK). Eluents were monitored with a Perkin-Elmer (Beaconsfield, UK) fluorescence detector and a computer (Dell Computer, Swindon, UK) was used to control the system. Chromatograms were run at 35 °C on a steel cartridge column (125x 3 mm ID) with a precolumn (4 x 4 mm ID) packed with 3μm Hypersil BDS C-18 (Hewlett-Packard, Stockport, UK). A flow rate of 0.45 ml/minute at 2400 psi was used. Slovents A and B were filtered through 0.22-μm filter (Millipore, Amicon, Stonehouse, UK) before use. The gradient program used was 42% A and 52% B (0-7 minutes), 35% A and 65% B (7-13 minutes), 25% A and 75% B (13-16 minutes), 0% A and 100% B (16-23 minuets, and 42% A and 58% B (23-28 minutes). This gradient program allowed the optimal separation of amino acids and internal standard (L-norvaline) from other endogenous amino acid in the CSF. The column eluent was monitored at excitation 340 mm, emission 440 nm. Precolumn derivatization of amino acid with OPA reagent, control of the HPLC system, calibration curves, and concentration calculations were automatically undertaken using the Unipoint (Anachem) software. 10μL CSF and 10 μL OPA were injected automatically into the HPLC for analysis. A typical chromatograph is shown in Figure 2.8.

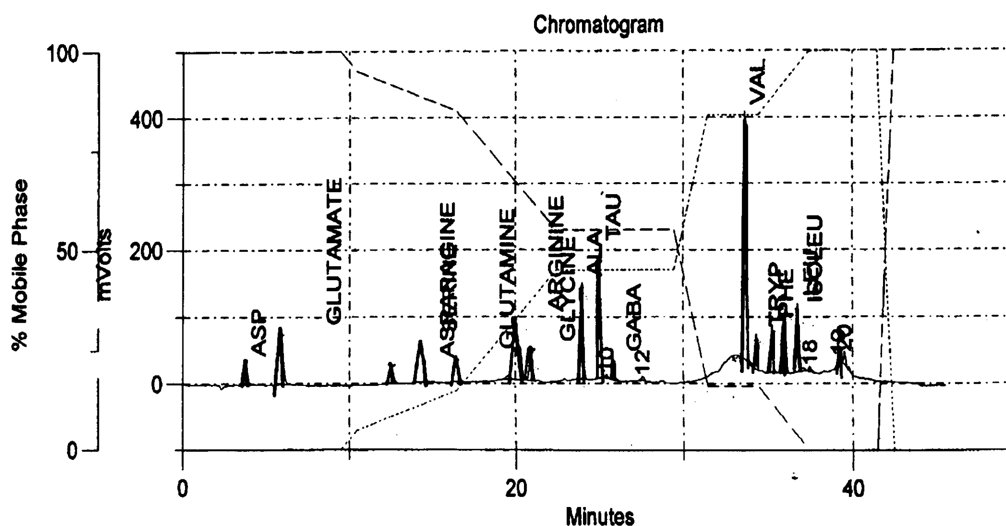


Figure 2.8: A typical HPLC chromatogram of amino acids measured in CSF. (Ala: alanine; Arg: arginine; Asn: asparagine; Asp: aspartic acid; Glu: glutamate; Gly: glycine; Lle: lsoleucine; Leu: leucine; Phe: phenylalanine; Ser: serine; Tau: taurine; Thr: threonine; Trp: tryptophan; Tyr: tyrosine; Val: valine; HMC: homocarnosine).

2.3.4.2 Preparations of buffer and standard solutions

Mobile-phase solvent A (pH 6.5) comprised of 250 mM phosphate, acetonitrile and HPLC grade water (32:9.5:58.5, vol:volvol) and mobile-phase solvent B comprised of acetonitrile, methanol and HPLC: grade water (28:24:28, vol/vol/vol). Borate buffer was prepared by adjusting boric acid (0.4M) to pH 10.5 with 5 M sodium hydroxide solution. The OPA derivatization reagent was prepared by dissolving 30 mg OPA in 0.4 ml methanol and adding 3.6 ml borate buffer and 50 μl 2-mercaptoethanol. A stock solution of amino acid standard (10 mM) was prepared using 0.1% HCl. Calibration standards comprised of 2.5, 5, 10, 15, 30 and 50 μmol/L concentration of the various amino acids. QC samples were prepared so as to contain 3, 20, 40 μmol/L of the various amino acids. CSF amino acids measured included: alanine, arginine, aspartic acid, asparagine, glutamate, glutamine, glycine, leucien,

lycine, homocarnosine, phenylanine, serine, taurine, threonine, trytophan, tyrosine and valine.

2.3.5 Measurement of microdialysate (ECF) amino acids

Amino acid concentrations in microdialysate from the frontal cortex and hippocampus after vigabatrin and levetiracetam administration were determined by HPLC and were measured by Dr. M. O'Connell (Cambridge Brain Repair Centre). Microdialysate amino acids measured include: arginine, aspartic acid, asparagine, citrulline, GABA, glutamate, glutamine, glycine, isoleucine, leucine, lycine, methionine, phenylanine, serine, taurine, threonine, tryptophan, tyrosine and valine.

2.3.5.1 HPLC instrumentation and chromatographic conditions

Five μL of dialysate were used for precolumn derivatization of amino acids by means of an OPA/3-mercaptoptopionic acid (MPA) reaction. Derivatization was conducted using an autoinjector, and the dialysate/reagent mixture was injected onto the HPLC system via a valve fitted with a 2 μL sample loop. The HPLC system consisted of two high pressure pumps with a gradient controller and mixing chamber (Micro-tech Scientific, Aunnyvale, USA). Separation of amino acids was achieved using an Amino Quant column and guard cartridge (HP, Cheshire, UK) placed inside a column oven operating at 40°C, using a linear gradient (5 to 100% elution mobile phase B in 18 minutes). The detector was a Gilson model 122 fluorometer (Gilson, Middleton, USA) fitted with a 5 μL flow cell and was operated with excitation and emission wavelengths of 340 and 450 nm respectively. Figure 2.9 shows a typical amino acid chromatogram.

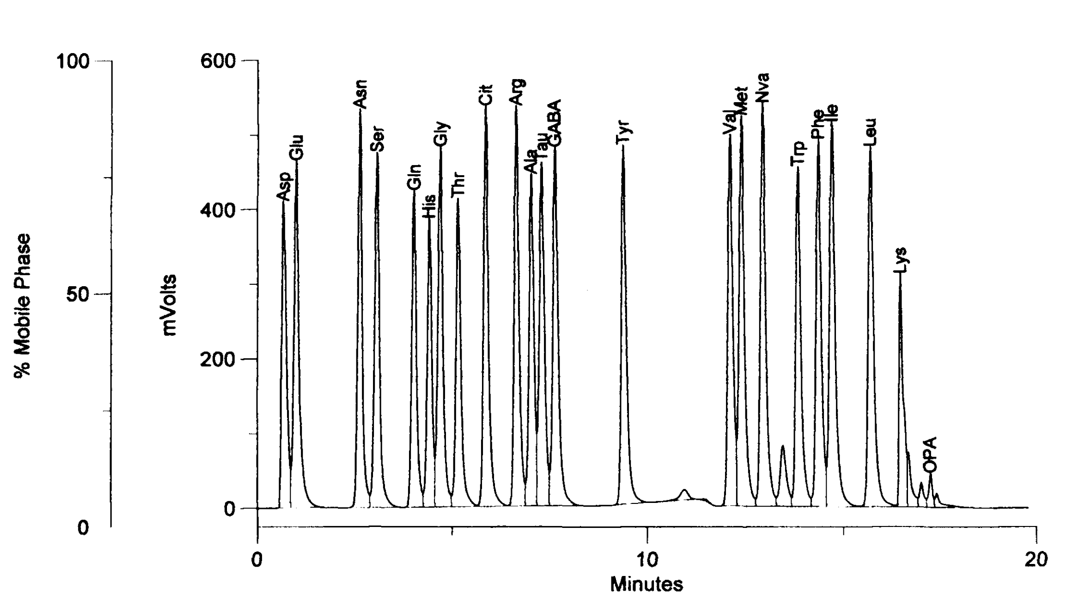


Figure 2.9: A typical HPLC chromatogram of amino acids measured in dialysate (Ala: alanine; Arg: arginine; Asn: asparagine; Asp: aspartic acid; Cit: citrullilne; Gln: glutamine; Glu: glutamate; Gly: glycine; Lle: lsoleucine; Leu: leucine; Lys: lysine; Met: Methionine; Phe: phenylalanine; Ser: serine; Tau: taurine; Thr: threonine; Trp: tryptophan; Tyr: tyrosine; Val: valine).

2.3.5.2 Preparations of buffer and standard solutions

Mobile phases A and B were prepared exactly as described in the AminoQuant method (Agilent Technologies UK Limited, West Lothian, UK). Calibration of the system was achieved using aqueous working standards prepared by dilution of an amino acid standard mixture (Agilent Technologies UK Limited, West Lothian, UK) following the additions of L-asparagine, L-glutamine, citrulline, GABA, norvaline (internal standard) and L-tryptophan. Calibration curves were constructed to cover the ranges 0.005 to 200 μM for each amino acid.

2.4 PROCEDURES FOR STIMULATION AND RECORDING OF PAIRED-PULSE INHIBITION

2.4.1 Electrode construction

All parts, unless otherwise specified, were purchased from Plastics One Inc via Semat Technical, Herts, UK. Wire was purchased from Advent Research Materials, Suffolk, UK. Bipolar stimulating electrodes were constructed from two 125 μm teflon coated stainless steel wires twisted together that were then attached to gold female connectors by soldering. The gold connectors could be mounted in a six holed plastic pedestal along with the connectors for the recording electrode and earth. The tips of the electrode were cut such that they were separated by 0.5 mm. The construction of the recording electrode was identical except that only one wire was used for unipolar recording, the other wire gave the electrode stability. Later experiments improved this set up by using a single 250 μm electrode coated stainless steel wire as the recording electrode. The earth was an electrode silver wire, which in the freely-moving experiments was connected to an anchor screw. For the freely-moving experiments the electrodes were kept in place using dental cement (De Trey, Surrey, UK) and three anchor screws.

2.4.2 Stimulator

Constant current stimuli were delivered from a Neurolog stimulator (NL304 period generator, NL 301 pulse generator, NL 505 flip-flop, NL 800 stimulus isolators: Digitimer Ltd, Welwyn Garden City, UK) as shown in Figure 2.10.

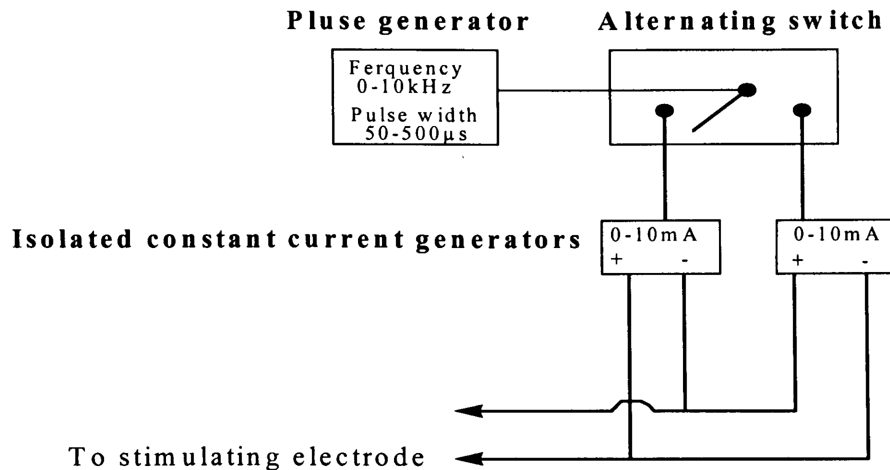


Figure 2.10: Stimulator arrangement: The pulse width could be set at 50, 150 or 500 μs; the frequency of pulses could be set from 0-10kHz; the amplitude could be set from 0-10mA, and using the alternating switch the polarity of the pulses from the constant current generators could be alternated (Walker, 1997).

The pulse generator could be gated so that the pulses could be synchronised with the computer recording. This gating also enabled fixed length trains to be generated, and using a purpose built series of flip-flop circuits, it was possible to generate fixed length trains that occurred at fixed intervals.

2.4.3 Recording equipment

Potentials were amplified and filtered (0.1 Hz to 5 kHz band pass) via a Neurolog amplifier (NL 100 A pre-amp, NL 104 A amplifier, NL 125 filter: Digitimer Ltd, Welwyn Garden City, UK) onto a storage oscilloscope (Figure 2.11).

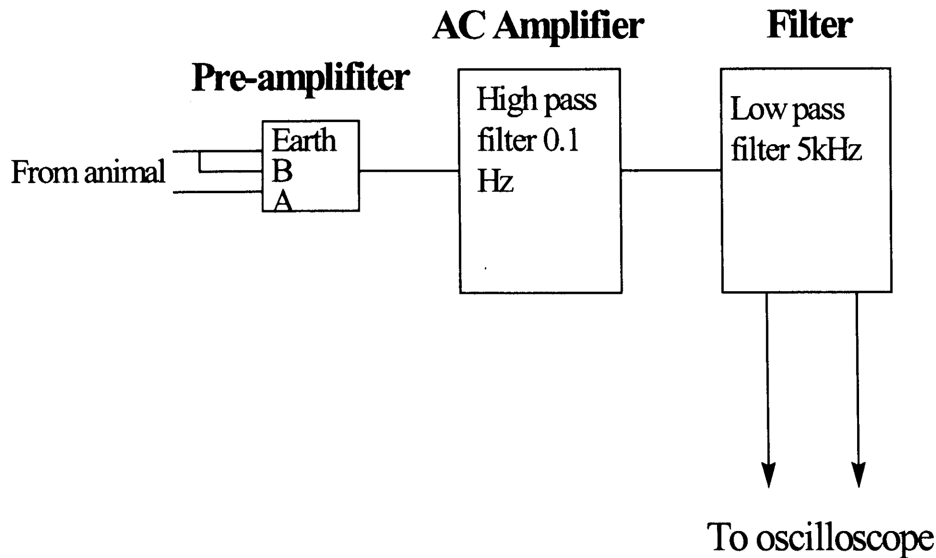


Figure 2.11: Amplifier arrangement. The amplifier was a differential AC amplifier with B connected to earth, and a built in high pass filter set at 0.1 Hz. A low pass filter set at 5kHz was also necessary to reduce high frequency interference that seemed to be due to computer equipment. Then the signal was amplified and pass to oscilloscope.

2.4.4 Electrode implantation

The animals were fully anaesthetised with 1-2 % halothane in oxygen as previously described. A subcutaneous silver electrode was used as earth. A recording electrode was implanted stereotaxically into the hippocampus (coordinates, 2.5 mm lateral, 4 mm posterior from bregma). A twisted (teflon- coated) stainless steel wire was advanced into the angular bundle (co-ordinates, 4.4 mm lateral, 8.1 mm posterior to bregma) to stimulate the perforant path.

Unipolar recordings were made from the recording electrode and potentials were amplified and filtered (0.1 Hz to 5 kHz band pass) via a Neurolog amplifier (Digitimer Ltd, Welwyn Garden City, U.K.) onto a storage oscilloscope and then stored on a computer using Labview 5.0 (National Instruments, Berkshire, U.K.).

The stimulating electrode was advanced 3-4 mm ventral from the dural surface. The recording electrode was lowered until the electrode entered the granule cell layer of the dentate. The depths of the electrodes were adjusted to maximise the slope of the population EPSP.

During field EPSPs, positive charge flows into dendrites resulting in a decrease in the potential of the fluid surrounding dendrites and charge flows out of soma and axons resulting in an increase in the potential surrounding soma and axons. Thus, in the dendritic region the field potential from neuronal depolarisation is negative with respect to surrounding tissue, whilst in the cell layer the field potential is positive with respect to surrounding tissue. If the intracellular depolarisation is large enough to result in an action potential, then the axon becomes very permeable to sodium and sodium ions flow into the axon and the circuit is reversed. This results in typical dentate granule cell field potentials (Figure 2.12 and 2.13)

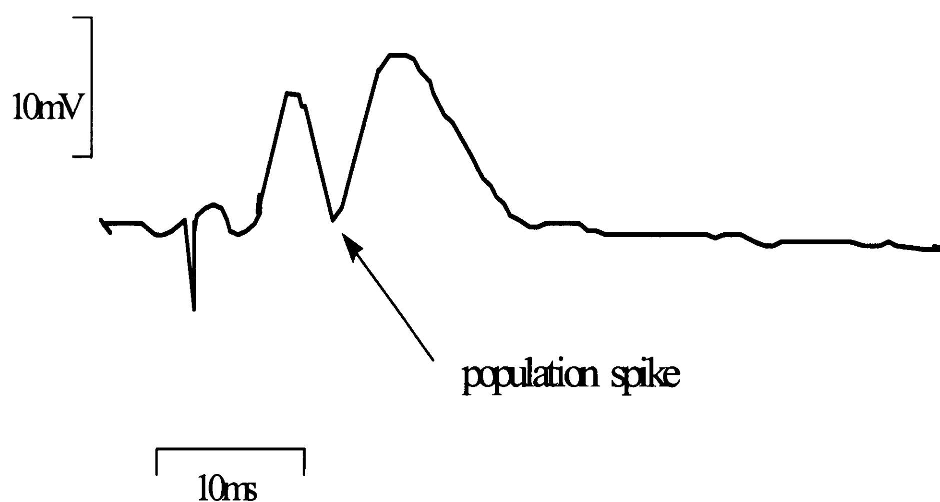


Figure 2.12: Field potential recorded from dentate granule cell layer following single pulse stimulation of the perforant path.

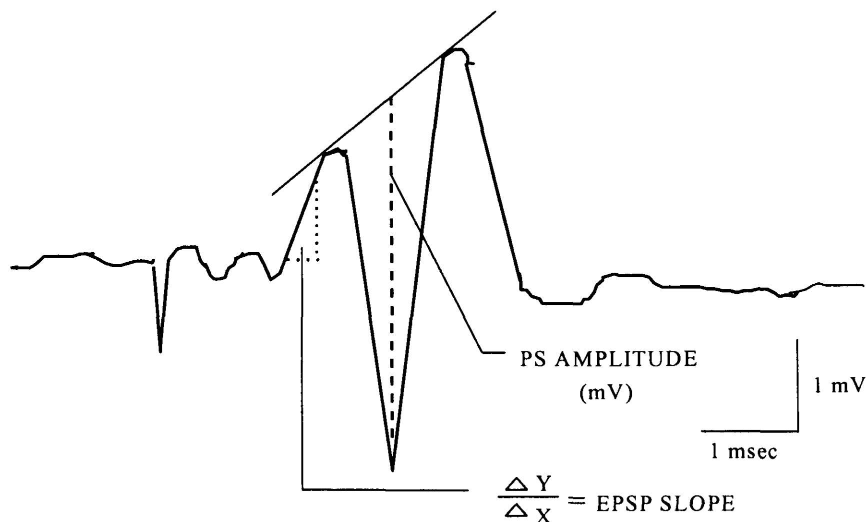


Figure 2.13: Measurement of the dentate gyrus field response components. Response recorded from the dentate gyrus after perforant path stimulation shows stimulus artifact (leftmost deflection) followed by a small fibre response and a large EPSP (positive wave) and population spike (negative wave) response. EPSP amplitudes were determined as the maximum rate of rise of the EPSP between its onset and the onset of the population spike (PS). PS amplitudes were measured from the extrapolated EPSP baseline to the peak of the PS waveform (dotted). EPSP and PS thresholds were defined as the lowest stimulus intensities producing an identifiable response (Joy & Albertson, 1992).

Once the recording electrode was positioned in the dentate granule cell layer, the depth of the stimulating electrode was adjusted to maximise the slope of the population EPSP. Constant-current stimuli were delivered from a Naurolog stimular (Digitimer Ltd, Welwyn Garden City, U.K.). Test shocks consisted of 1-2mA, 50 μs monopolar pulses every 10s. The recording electrode was lowered until it entered the cell layer of dentate.

2.4.5 Paired-pulse stimulation protocols

Three days after recovery from anaesthesia, the perforant path was stimulated with 3-6mA, 50 μs monopolar paired pulses. The stimulus strength was adjusted in order to achieve a population spike that was 50 % of maximum. Paired pulses were delivered at a frequency of 0.2 Hz with alternating paired pulse intervals of 20, 50 and 100 ms. A baseline was recorded for 30 minutes prior to administrations. The rats were then administered with either 1000 mg/kg vigabatrin or 80mg/kg levetiracetam i.p. Immediately, paired pulse recordings were made for 5 minutes every 30 minutes until 8 hours after vigabatrin and levetiracetam administration. A further recording was made 24 hours later. Because paired pulse inhibition is dependent on the size of the population spike resulting from the conditioning pulse, the stimulus amplitude was adjusted to maintain a constant conditioning population spike.

2.5 STATISTICS

2.5.1 Pharmacokinetic and neuropharmacokinetic data

Kinetic parameters were calculated using each individual animal at each time point. The concentration versus time profiles for serum, CSF and ECF were analysed according to a one compartment model using Statistica 5. Time to maximum plasma concentration (T_{max}) and the maximum concentration (C_{max}) were estimated from the concentration versus time plots. The area under the plasma concentration versus time curve (AUC) to the last plasma concentration was obtained using the linear trapezoidal rule for t=0-x (where x=last time point) and was extrapolated to infinity by calculating $AUCx\text{-}\infty = C(x)/\beta$. The elimination half-life

($t_{1/2}$) was calculated from the slope (λz), estimated by log linear regression of the terminal phase of the plasma concentration-time curve. Results are presented as mean ± sem. Data were compared using Student' *t*-test.

2.5.2 CSF and ECF amino acids

Amino acid concentration after administration of vigabatrin or levetiracetam at each time point was compared to the 3 baseline points or control group. The results were generalised, and were presented as mean ± sem. Data were compared using Student's *t*-test and ANOVA for dialysate amino acids and Student's t-test for CSF amino acids. The Student's t-test used the mean of three baseline data which were compared to each subsequent time point and ANOVA (single factor) used AUC summary data to analyse serial measurements and to compare the drug to control group at each time point.

2.5.3 Paired-pulse inhibition data

From each 5 minute epoch, paired pulses were chosen in which the conditioning population spike (p1) remained within 10 % of the amplitude of the baseline conditioning population spike. Averages were taken of these paired pulses, and paired pulse inhibition was expressed as the amplitude of the population spike following the test pulse (p2) divided by the amplitude of the population spike following the conditioning pulse x 100.

Chapter 3

Pharmacokinetics and neuropharmacokinetics of vigabatrin

3.1 INTRODUCTION

The action of a drug is dependent on its pharmacokinetics. It is therefore important to understand the pharmacokinetics of a drug and, in particular, its concentration at its site of action. Although there have been numerous studies reporting the peripheral (blood) pharmacokinetics and central (CSF and ECF) neuropharmacokinetics of vigabatrin in man (Haegele & Schechter, 1986; Hoke et al, 1993; Schechter, 1989; Ben-Menachem, 1995, Ben-Menachem et al, 1988 & 1989; Durham et al, 1993; Grove et al, 1984 & 1981) and in animal models (Jung et al, 1977; Smithers et al, 1985; Bernasconi et al, 1988; Patsalos & Duncan, 1995), these have usually involved composite values derived from single time point determinations. Furthermore, the inter relationship between the peripheral and central kinetics of vigabatrin has not been systematically investigated. In this chapter the inter-relationship of vigabatrin pharmacokinetics in blood (serum), neuropharmcokinetics in CSF and brain ECF (frontal cortex and hippocampus) were investigated after intraperitoneal administration of vigabatrin in the freely behaving rat.

3.2 EXPERIMENTAL PROTOCOL

Male Sprague-Dawley rats (Charles River, Margate, Kent) weighing 300-350 g were used. Two types of experiments were undertaken. In the first, a blood catheter and a CSF cannulae were surgically implanted into the right jugular vein and cisterna magna respectively. In the second, a blood catheter was implanted into the right jugular vein and two microdialysis probes (one in the cerebral cortex and the other in the hippocampus) were surgically implanted. The surgical procedures are described in Chapter 2. Two days after recovery from surgery, baseline CSF and blood samples were

taken and the cannulated animals were administered by intraperitoneal injectionwith 250, 500 and 1000 mg/kg vigabatrin for serum and CSF sampling and 500 and 1000mg/kg for ECF sampling. Venous blood samples (100μl) were withdrawn every 20 minutes for the first hour and then at 30 minute intervals for a further 7 hours. CSF samples (15 μL) were collected at 30 minute intervals for 8 hours and dialysate samples (20 μL) were collected every 20 minutes for 4 hours and subsequently at 30 minute intervals for a further 4 hours. Vigabatrin concentrations were determined in serum, CSF and ECF as described in Chapter 2.

3.3 RESULTS

3.3.1 Serum pharmacokinetics

The vigabatrin serum concentration versus time profiles after 250, 500 and 1000 mg/kg vigabatrin administrations are shown in Figure 3.1. Intraperitoneally administered vigabatrin demonstrated rapid absorption. Peak vigabatrin concentrations (*T*max) were achieved at 0.4h ± 0. 06 (mean ± sem) and were dose independent. Subsequently, vigabatrin concentrations gradually declined and the elimination half-life was 1.1± 0.1 hours at the lower doses, and 1.4 ± 0.1 hours at the higher dose. Table 3.1 shows the apparent pharmacokinetic constants for individual rats together with the mean values as calculated from the concentration versus time plots. The pharmacokinetic constants for individual rats showed some variability within each dose group. The mean values of C_{max} and AUC for vigabatrin increased proportionately with increasing dose. Half-life values were dose independent and it was prolonged at the higher dose ($p < 0.05$). Since C_{max} and T_{max} values were determined by visual inspection of the concentration versus time curve and as the first sampling time point was at 20 minutes,

these values may have been under-estimated and over-estimated respectively.

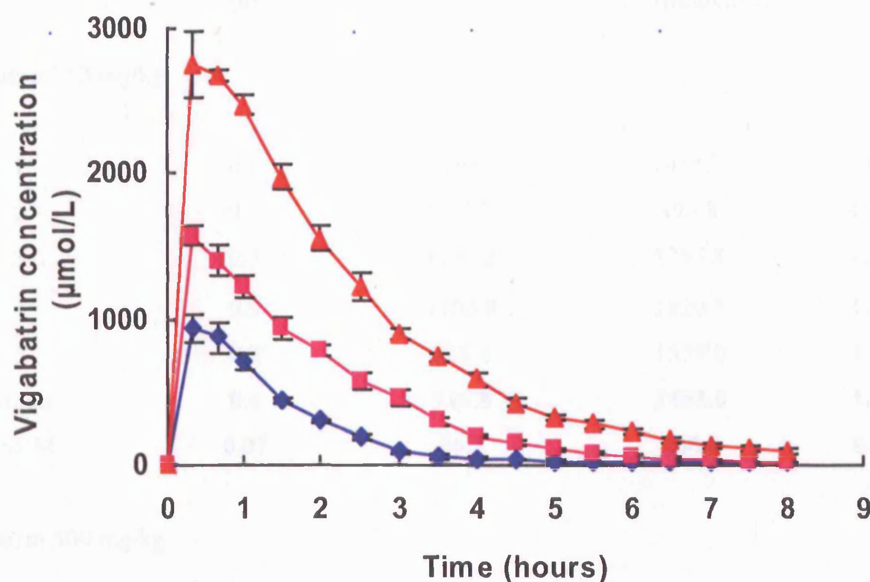


Figure 3.1: Serum concentration versus time profiles for vigabatrin after 250mg/kg (◆), 500 mg/kg (■) and 1000 mg/kg (▲) vigabatrin intraperitoneal administration. Data are mean ± sem of 5-7 animals.

The free non-protein bound vigabatrin concentration was determined from 9 samples that spanned the concentration range and the time range studied in this study. Figure 3.2 shows the free/total vigabatrin concentration ratio for the 9 samples and the calculated mean free fraction was 0.99 ± 0.01. These data indicate that vigabatrin is not protein bound.

Table 3.1: Serum vigabatrin pharmacokinetic constants after intraperitoneal administration of 250, 500 and 1000 mg/kg vigabatrin

Rat No.	T_{max} (h)	C_{max} (μmol/L)	AUC (μmol h/L)	$t½$ (h)
Vigabatrin 250 mg/kg				
1	0.3	1030.7	1455.7	1.0
2	0.7	585.7	893.8	1.2
3	0.3	1101.2	1765.8	1.3
4	0.3	1105.8	1820.5	1.2
5	0.3	925.8	1539.0	1.0
Mean	**0.4**	**949.8**	**1495.0**	**1.1**
±SEM	**0.07**	**96.7**	**166.0**	**0.1**
Vigabatrin 500 mg/kg				
1	0.3	1803.8	4022.3	0.8
2	0.3	1578.4	3276.9	1.4
3	0.3	1528.5	3634.9	1.2
4	0.3	1289.4	2786.5	1.1
5	0.3	1558.7	3275.1	1.2
6	0.7	1814.2	3507.1	1.0
Mean	**0.4**	**1595.5**	**3417.1**	**1.1**
±SEM	**0.06**	**79.8**	**169.2**	**0.1**
Vigabatrin 1000 mg/kg				
1	0.3	2744.1	6311.0	1.6
2	0.3	3412.5	7818.4	1.6
3	0.3	3310.8	8394.8	1.7
4	0.3	2992.3	6871.7	1.4
5	0.7	2733.8	7169.2	1.3
6	0.7	2581.0	7394.5	1.5
7	0.3	2815.0	6914.6	1.0
Mean	**0.4**	**2941.3**	**7267.7**	**1.4**
±SEM	**0.06**	**118.4**	**258.0**	**0.1**

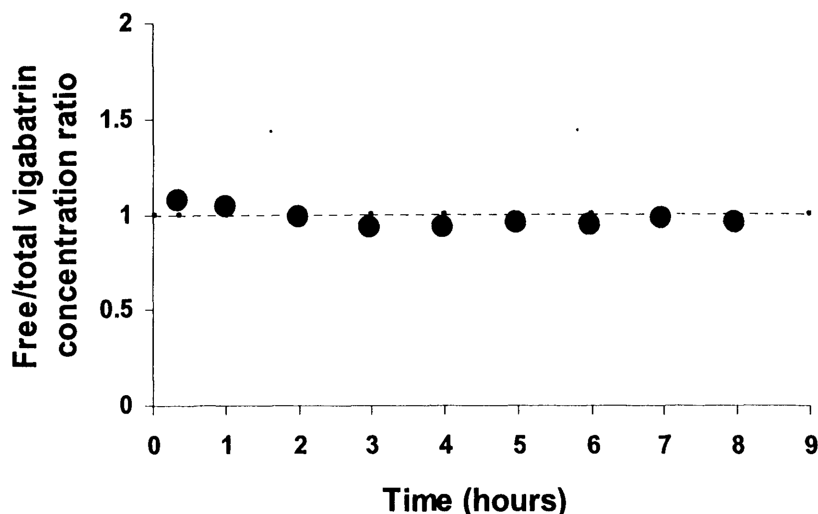


Figure 3.2: Vigabatrin free/total concentration ratios versus time profile.

3.3.2 CSF neuropharmacokinetics

Figure 3.3 shows the CSF concentration verses time profiles after intra-peritoneal administration of 250, 500 and 1000 mg/kg vigabatrin. It can be seen that vigabatrin was detectable in the CSF compartment at the first sampling time point (30 minutes). The time to the peak concentration (T_{max}) occurred at approximately 1 hour after vigabatrin administration, and then fell exponentially. CSF vigabatrin concentrations rose linearly and dose-dependently. The apparent neuropharmacokinetic constants for individual rat together with the mean values as calculated from the concentration versus time curves are shown in Table 3.2. Mean T_{max} values ranged from 0.8-1.2 hours and mean half-life values were 2.0 h, 3.2 h and 3.3 h for 250, 500 and 1000mg/kg vigabatrin administration respectively (Table 3.1 & 3.2). However, whilst half-life values were significantly different ($P < 0.005$) between 250 mg/kg to 500 mg/kg and between 250 mg/kg and 1000 mg/kgvigabatrin administration, there was no

difference between 500 and 1000 mg/kg vigabatrin administration. Half-life values were also significantly longer than those values in the serum ($P < 0.001$) at all doses.

Table: 3.2: Neuropharmacokinetic constants after intraperitoneal CSF vigabatrin administration of 250, 500 and 1000mg/kg vigabatrin

Rat No.	T_{max} (h)	C_{max} (µmol/L)	AUC (µmol h/L)	$t_{½}$ (h)
Vigabatrin 250 mg/kg				
1	1.0	19.5	61.7	1.9
2	1.5	21.9	56.6	1.3
3	1.0	17.8	83.8	2.7
4	2.0	15.2	72.8	2.7
5	1.0	22.4	85.0	2.4
6	0.5	14.8	68.1	2.1
Mean	**1.2**	**18.6**	**71.8**	**2.2**
±SEM	**0.2**	**1.3**	**4.4**	**0.2**
Vigabatrin 500 mg/kg				
1	0.5	20.7	95.8	3.4
2	1.0	30.4	108.2	2.8
3	1.0	46.6	189.6	2.8
4	1.0	27.3	99.8	4.3
5	0.5	36.4	130.8	2.5
6	1.0	29.5	114.0	3.2
Mean	**0.8**	**31.8**	**123.0**	**3.2**
±SEM	**0.1**	**3.6**	**14.2**	**0.3**
Vigabatrin 1000mg/kg				
1	1.0	37.2	180.6	3.5
2	1.0	31.1	129.4	2.9
3	1.0	80.1	412.7	3.6
4	1.0	47.0	267.4	4.1
5	0.5	45.2	190.8	2.9
6	0.5	63.3	239.1	2.9
Mean	**0.8**	**50.6**	**236.7**	**3.3**
±SEM	**0.1**	**7.4**	**40.3**	**0.2**

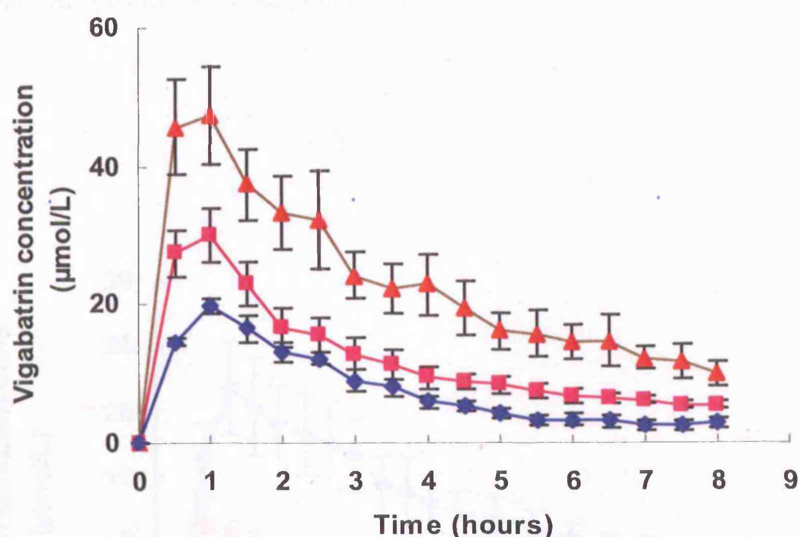


Figure 3.3: CSF concentration versus time profiles for vigabatrin after intraperitoneal administration of 250mg/kg (◆), 500mg/kg (■) and 1000mg/kg (▲) vigabatrin. Data are mean ± sem of 6 animals.

3.3.3 ECF neuropharmacokinetics

The mean ± SEM *in vitro* relative recovery for vigabatrin from 28 microdialysis probes was 21.69 ± 1.46 % at a dialysate flow rate of 1 µL/min. These data were used to adjust the *in vivo* concentration data accordingly.

Following 500 mg/kg vigabatrin administrated intraperitoneally, vigabatrin was rapidly detected in the dialysate in both the frontal cortex and hippocampus (Figure 3.4 A). Vigabatrin concentration peaked at approximately 1 hour post vigabatrin administration and then declined exponentially. A similar pattern was observed following 1000 mg/kg vigabatrin administration (Figure 3.4 B). Vigabatrin was also rapidly detected in the dialysates of both the frontal cortex and the hippocampus. Vigabatrin concentration peaked at 0.8 hour and then declined exponentially. From figure 3.5 it can be seen vigabatrin concentrations increased in the frontal cortex (A) and

the hippocampus (B) in a dose dependent manner.

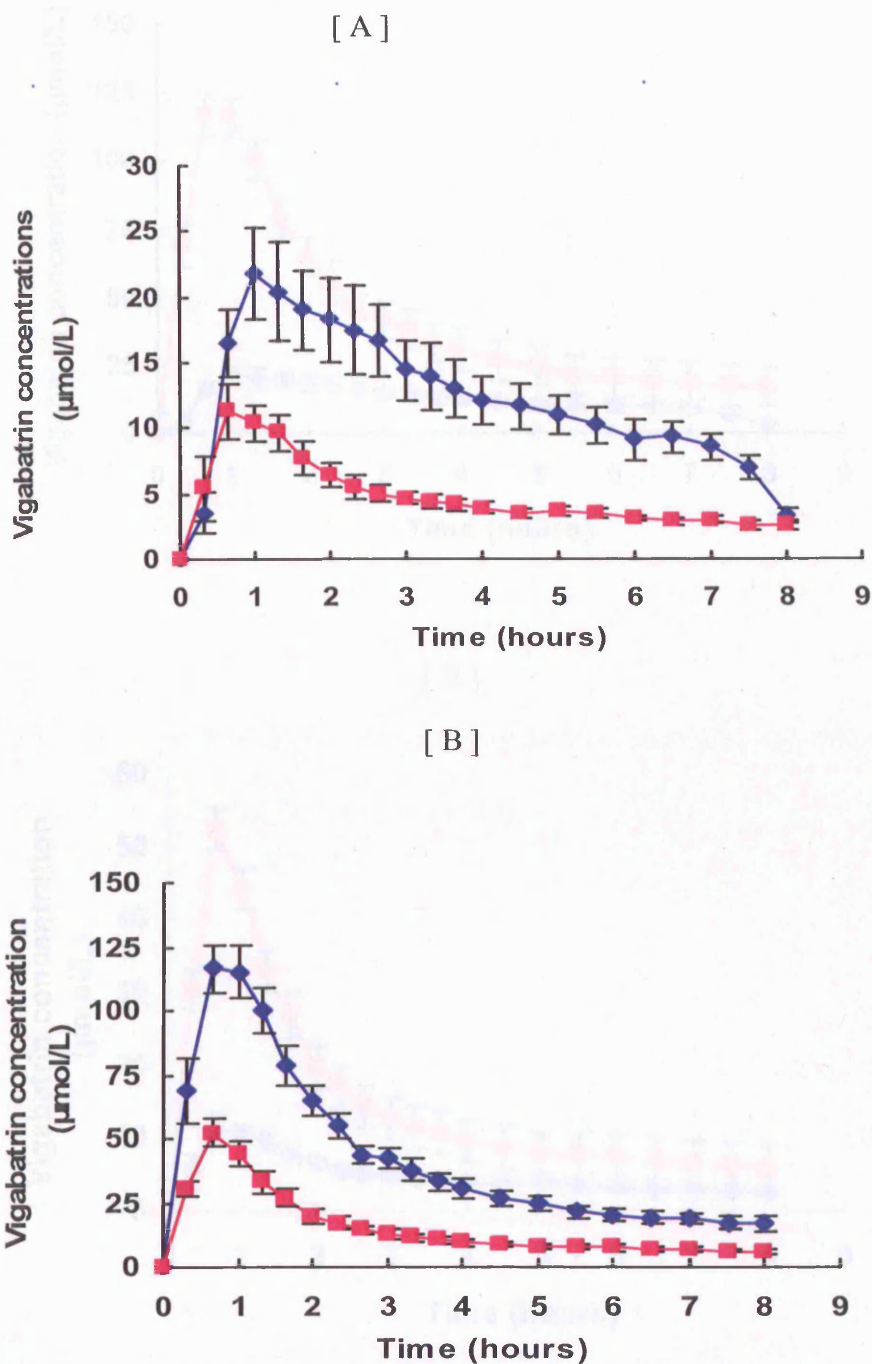
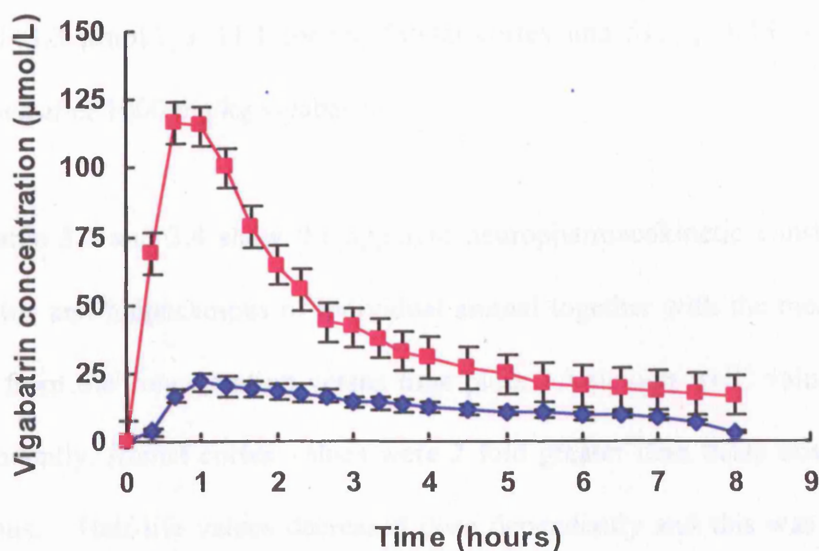


Figure 3.4: Vigabatrin concentration versus time profiles for frontal cortex (◆) and for hippocampus (■) after intraperitoneal administration of vigabatrin (A) 500 mg/kg and (B) 1000 mg/kg. Values are mean ± sem of 7 rats.

[A]



[B]

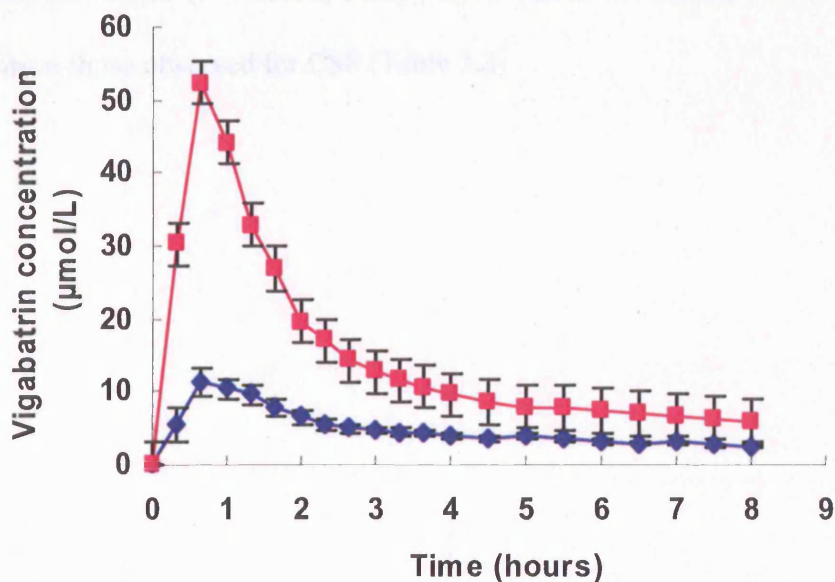


Figure 3.5: Vigabatrin concentration versus time profiles for the frontal cortex (A) and the hippocampus (B) after intraperitoneal administration of vigabatrin 500 mg/kg (◆) and 1000 mg/kg (■). Values are mean ± sem of 7 animals.

C_{max} values in the frontal cortex were greater than those in hippocampus and

this occurred for both doses. Vigabatrin concentrations were 22.5 μmol/L ± 3.2 for the frontal cortex and 12.7 μmol/L ±1.6 for the hippocampus after 500 mg/kg vigabatrin, and were 113.3 μmol/L ± 11.1 for the frontal cortex and 51.7 μmol/L ± 4.8 for the hippocampus after 1000 mg/kg vigabatrin.

Table 3.3 and 3.4 show the apparent neuropharmacokinetic constants for the frontal cortex and hippocampus of individual animal together with the mean values as calculated from the concentration versus time plots. Although AUC values increased dose dependently, frontal cortex values were 2 fold greater than those observed in the hippocampus. Half-life values decreased dose dependently and this was observed to occur in both the frontal cortex and the hippocampus (this contrasts with the dose-halflife relationship in serum and CSF). These values of half-life in brain ECF were significantly prolonged ($P < 0.001$) compared to that in the serum, but values were not different from those observed for CSF (Table 3.2)

Table 3.3: Neuropharmacokinetic constants for frontal cortex ECF after intraperitoneal administration of 500 and 1000 mg/kg vigabatrin

Rat No.	T_{max} (h)	C_{max} (µmol/L)	AUC (µmol h/L)	$t_{½}$ (h)
Vigabatrin 500 mg/kg				
1	0.7	20.5	83.3	3.0
2	1.0	24.9	112.6	3.0
3	0.7	13.7	90.2	3.8
4	0.7	23.5	90.5	2.7
5	1.3	14.4	95.9	4.5
6	1.7	21.1	145.2	4.7
7	1.3	39.4	194.4	3.0
Mean	**1.0**	**22.5**	**115.9**	**3.6**
±SEM	**0.1**	**3.2**	**15.3**	**0.3**
Vigabatrin 1000 mg/kg				
1	0.7	142.5	284.8	1.9
2	0.7	132.9	540.4	3.3
3	0.7	70.7	531.8	2.2
4	0.7	108.3	421.1	3.1
5	0.7	148.7	334.6	2.2
6	0.7	114.4	408.0	2.6
7	1.0	96.5	349.5	2.3
Mean	**0.7**	**113.4**	**410.0**	**2.5**
±SEM	**0.1**	**10.1**	**36.8**	**0.2**

Table 3.4: Neuropharmacokinetic constants for hippocampal ECF after intraperitoneal administration of 500 and 1000 mg/kg vigabatrin

Rat No.	T_{max} (h)	C_{max} (μmol/L)	AUC (μmol h/L)	$t_{½}$ (h)
Vigabatrin 500 mg/kg				
1	1.0	10.0	48.9	4.6
2	0.7	13.9	53.3	4.0
3	0.7	19.9	63.7	3.4
4	1.0	10.4	48.2	3.0
5	1.0	14.5	41.8	2.5
6	1.0	13.8	57.8	3.3
7	0.7	6.2	30.4	4.5
Mean	**0.9**	**12.7**	**49.2**	**3.6**
±SEM	**0.1**	**1.6**	**4.1**	**0.3**
Vigabatrin 1000 mg/kg				
1	1.0	44.8	160.0	3.1
2	0.7	41.7	72.0	1.4
3	1.0	54.7	123.0	2.1
4	0.7	33.4	121.7	3.4
5	0.7	68.8	190.2	2.9
6	0.7	52.6	143.6	2.2
7	0.7	66.4	141.3	1.8
Mean	**0.8**	**51.7**	**135.9**	**2.4**
±SEM	**0.1**	**4.9**	**13.9**	**0.2**

3.3.4 Comparison of serum, CSF and ECF kinetic parameters of vigabatrin

Figure 3.6 shows CSF/serum of vigabatrin concentration ratios after 250, 500 and 1000mg/kg vigabatrin administrations. Values rose gradually during first 7 hours post vigabatrin administration, and tended towards an equilibrium at 7-8 hours (as measured by a constant CSF/serum vigabatrin concentration ratio).

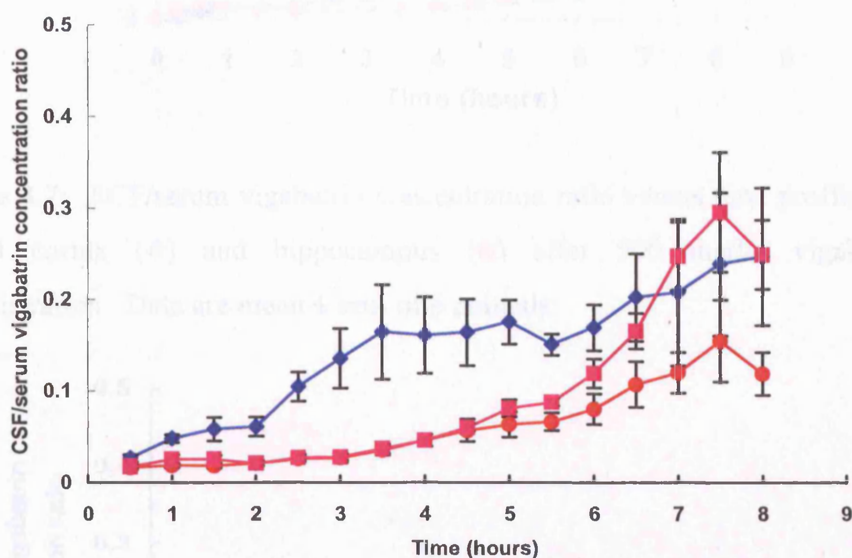


Figure 3.6: CSF/serum vigabatrin concentration ratio versus time profiles for 250 mg/kg (●), 500 mg/kg (◆) and 1000mg/kg (■) vigabatrin. Date are mean ± sem of 6 animal.

Figures 3.7 and 3.8 show the ECF/serum vigabatrin concentration ratios for the frontal cortex and hippocampus respectively after 500 and 1000mg/kg vigabatrin administrations. It can be seen that the ECF/serum ratios followed a similar pattern as for the CSF/serum ratios, rising slowly during the first 7 hours post vigabatrin administration, and with a tendency towards equilibrium at 7-8 hours (as measured by a content ECF/serum vigabatrin concentration ratio).

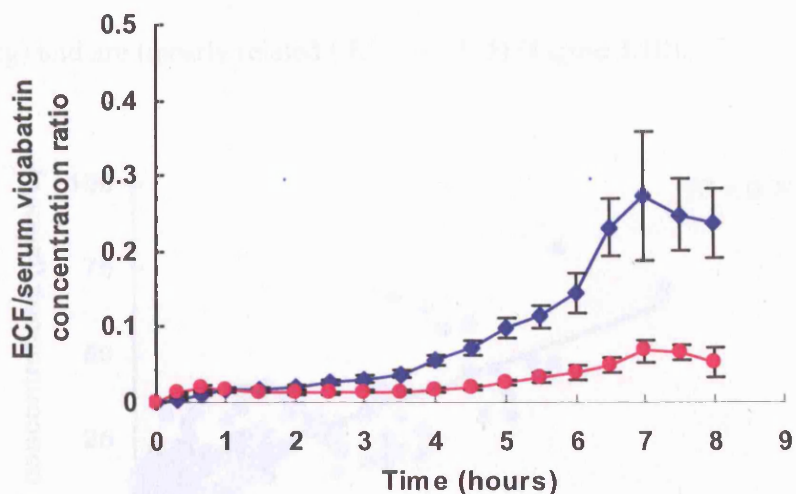


Figure 3.7: ECF/serum vigabatrin concentration ratio versus time profiles for frontal cortex (◆) and hippocampus (■) after 500 mg/kg vigabatrin administration. Data are mean ± sem of 6 animals.

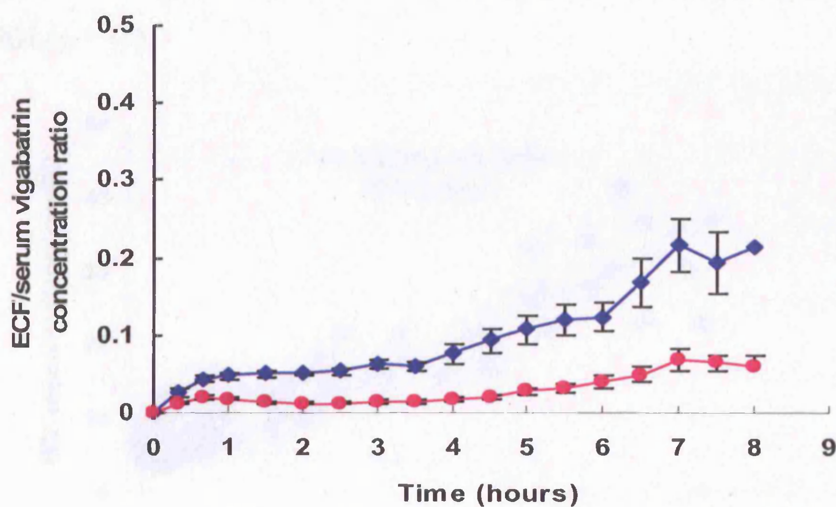


Figure 3.8: ECF/serum vigabatrin concentration ratio versus time profiles for frontal cortex (◆) and hippocampus (■) after vigabatrin 1000 mg/kg administration. Data are mean ± sem of 6 animals.

The relationship between vigabatrin concentration in serum and CSF after vigabatrin administration (250, 500 and 1000 mg/kg) is shown in Figure 3.9. It can be seen that the relationship is linear (R^2 = 0.704). Similarly, the relationship between

vigabatrin concentration in serum and ECF after vigabatrin administration (500 and 1000 mg/kg) and are linearly related ($R^2 = 0.9355$) (Figure 3.10).

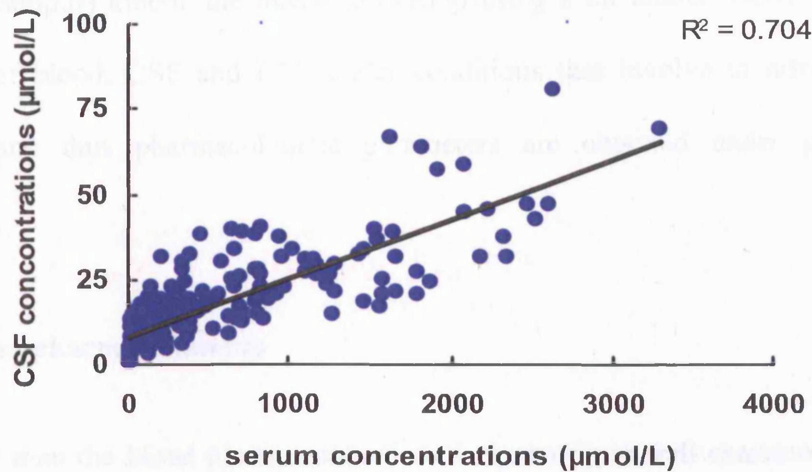


Figure 3.9: Relationship between serum and CSF concentration. $R^2 = 0.704$ (n = 17).

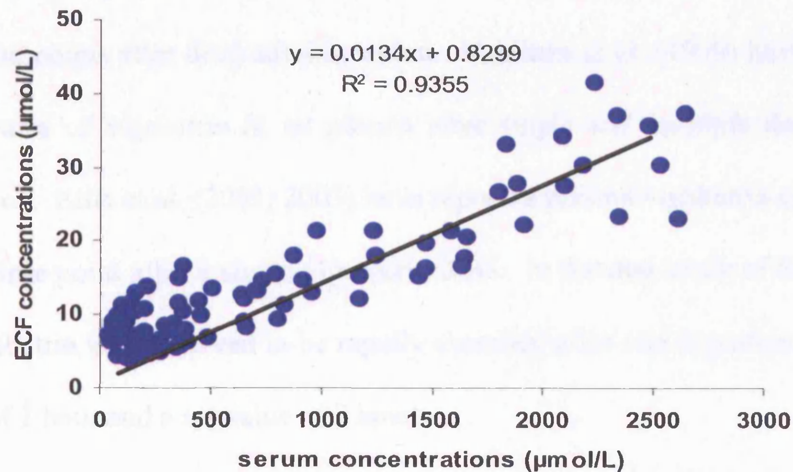


Figure 3.10: Relationship serum and ECF concentration. The equation for linear regression is $R^2 = 0.9355$ of the average in two doses (●).

3.4 DISCUSSION

The present study sought to investigate the blood, CSF and ECF (frontal cortex and hippocampus) kinetic the inter-relationship using a rat model which allows serial sampling of blood, CSF and ECF under conditions that involve in minimal animal handling and thus pharmacokinetic parameters are obtained under physiological conditions.

3.4.1 Serum pharmacokinetics

In man the blood pharmacokinetics of vigabatrin is well characterised (Rey et al., 1992). However, there is a sparcity of data on the blood pharmacokinetics of vigabatrin in animal species (Smithers et al., 1985) and those that are published in relation to the rat represent composite values obtained from individual animals killed at different time points after drug administration. Valdizan et al. (1999) have reported on the time course of vigabatrin in rat plasma after single and multiple dose vigabatrin administration. Sills et al. (2001, 2003) have reported plasma vigabatrin concentrations at a single time point after a single vigabatrin dose. In the dog study of Smithers et al. (1985), vigabatrin was observed to be rapidly absorbed after oral ingestion with a blood T_{max} value of 1 hour and a $t_{1/2}$ value of 7 hours.

In the present study vigabatrin was administered as a single dose (250, 500 or 1,000 mg/kg) by intraperitoneal injection and the major serum pharmacokinetic findings of this study are that: (1) the pharmacokinetics of vigabatrin in serum are dose-dependent; (2) vigabatrin T_{max} values (mean value 0.4 h) would suggest that vigabatrin is readily and quickly absorbed from the peritoneal cavity; (3) vigabatrin is not protein

bound in serum and therefore readily distributed and available for entry and distribution into the brain; (4) the elimination of vigabatrin from serum is rapid (mean $t_{1/2}$ values, 1.1-1.4 h) and there is dose independent at 250-500 mg, but demonstrates dose independence at higher doses (500-1000mg).

Although there are reports of single point (at 2 hours of 4 hours post-dose) concentration analysis of serum vigabatrin in the rat after acute administration of 250, 500 and 1000 mg/kg vigabatrin, such data do not allow any calculation of pharmacokinetic parameters (Sills et al., 2001, 2003). The study of Vladizan et al (1999) reported on the serum vigabatrin concentration versus time profile after a single intraperitoneal administration of 200 mg/kg vigabatrin. Blood samples were collected by heart puncture (thus requiring a single rat per time point) and samples were collected only at 4, 24, 48 and 72 hours post-dose. Although the calculated $t_{1/2}$ value from these data is 11 hours, the fact that vigabatrin was not detectable at 48 hours post-dose and that the $t_{1/2}$ value is based on only two time points (4 and 24 hours) would suggest that the value cannot be considered as accurate. In the present study mean $t_{1/2}$ values for serum vigabatrin, as calculated from vigabatrin concentration versus time profiles characterised by 17-time points over a sampling period of 8 hours, varied between 1.1 and 1.4 hours (Table 3.1).

3.4.2 CSF neuropharmacokinetics

As the CSF compartment is suggested to be kinetically indistinguishable from that of the biophase or site of brain action of drugs that are active in the brain, the present study used a well established and validated rat model so as to determine CSF

neuropharmacokinetics of vigabatrin after single dose vigabatrin (250, 500 and 1,000 mg/kg) administration.

In patients with epilepsy, analysis of CSF obtained by single lumber puncture after single dose vigabatrin ingestion has shown vigabatrin to be present at 6 hours post-ingestion and CSF vigabatrin concentrations to be approximately 10-15% of blood concentrations (Ben-Menachem 1989; Ben-Menachem et al., 1989). Vigabatrin concentrations in CSF increase dose-dependently and show a tendency to accumulate after multiple-doses (Riekkinen et al., 1989). In animal study, the time course of vigabatrin has also been reported in rat CSF (Halonen et al, 1990; 1991) and mouse brain (Jung et al., 1997; Sills et al., 2001; 2003).

In the present study, the major vigabatrin CSF neuropharmacokinetic findings after single-dose intraperitoneal vigabatrin administration (250, 500 or 1,000 mg/kg) are that: (1) vigabatrin rapidly (mean T_{max} values, 0.8-1.2 h) penetrated the blood-brain barrier and was detectable at the time of first CSF sampling (30 minutes post-dose); (2) although the CSF pharmacokinetics of vigabatrin paralleled that seen in serum, CSF vigabatrin concentrations represented only 3-5% of serum vigabatrin concentrations and did not reflect free drug concentrations in serum; (3) the efflux of vigabatrin from the CSF compartment was significantly slower (mean $t_{1/2}$ values, 2.2-3.3 h) than that suggested by serum values (mean $t_{1/2}$ values, 1.1-1.4 h).

That vigabatrin enters the CSF compartment was confirmed by Halonen et al (1990) who reported on cisternal CSF vigabatrin concentrations after a single vigabatrin dose (1,000 mg/kg) administered intraperitoneally. CSF samples were collected at 5, 24,

48 and 96 hours after vigabatrin administration and showed that vigabatrin concentrations were at their highest at 5 hours and interestingly vigabatrin was still measurable in CSF samples collected at 96 hours. Unfortunately, the data do not allow for any meaningful calculation of CSF pharmacokinetic data. However, comparing the CSF vigabatrin concentrations achieved at 5 hours post dose with concentrations observed in the present study (Figure 3.3) vigabatrin concentrations are significantly different (88 μmol/L versus 18 μmol /L for the present study). The fact that the two studies used different rat strains may in part explain the difference.

Two physiochemical characteristics of drug which determine the rate and degree of entry into the CSF and brain are lipid solubility and the degree of serum protein binding. Because vigabatrin has high water solubility, it would not be expected to gain rapid entry into the brain. Nevertheless, vigabatrin did rapidly enter the central compartment since vigabatrin was detectable in CSF at time of first sampling (30 minutes; Figure 3.3). Additionally, the rate of entry into the CSF was dose-dependent, as there was difference in T_{max} values after vigabatrin dose administration (250, 500 and 1,000 mg/kg). Indeed, serum and CSF vigabatrin concentrations were linearly related (Figure 3.9). These data suggest that vigabatrin may be actively transported into the brain.

As the serum protein binding of a drug is a determinant of the available drug that can enter the brain/CSF, it might be expected that vigabatrin, which is not protein bound in serum, would be located in high concentrations in these central compartments. However, this is not the case with CSF concentrations representing but a small fraction (3-5%) of the concentrations observed in serum. In man the equivalent figure is 10%

(Ben-Menachem et al., 1989). The reason for this is unknown, but these data with vigabatrin parallel that observed with another GABA acting drug, tiagabine (Wang et al., 2004), and would also be consistent with an active transport mechanism.

3.4.3 ECF neuropharmacokinetics

Although the CSF compartment is considered to be kinetically indistinguishable from that of the biophase or site of brain action of drugs that are active in the brain, some drugs (e.g. phenytoin) are known not to be uniformly distributed in the brain (Walker et al., 1996). Consequently, microdialysis monitoring of ECF in specific brain sites is considered to be a more appropriate index of events that occur at the synaptic level and thus at the site of drug action.

There have been no reports on vigabatrin ECF neuropharmacokinetics, although as highlighted earlier some composite brain data have been reported from which some tentative kinetic parameters can be deduced (Jung et al., 1997; Sills et al., 2001; 2003). In the present study the ECF neuropharmacokinetics of vigabatrin were simultaneously determined in the rat frontal cortex and hippocampus after 500 and 1000 mg/kg vigabatrin administration by intraperitoneal injection. Vigabatrin entered the ECF compartment rapidly with T_{max} values at 0.7-1.7 hours. However, vigabatrin distribution was brain region specific with vigabatrin concentrations in the frontal cortex being 2-fold greater compared with that in the hippocampus. This concentration difference was observed for both 500 mg/kg and 1000 mg/kg vigabatrin administration. Mean vigabatrin $t_{1/2}$ values were identical for frontal cortex (2.5-3.6 hours) and hippocampus (2.4-3.6 hours), which would suggest that the elimination processes in the two brain

regions are similar. There is no evidence that vigabatrin is transported by PGP. Vigabatrin is transported by amino acid transporters (Abbot et al, 2006). It is possible that vigabatrin is transported into brain. So the two explanations are either it is being pumped out faster (e.g. via PGP) or that it is being pumped in more efficiently. Since the rate of elimination is similar for frontal cortex and hippocampus, then the only explanantion is that there is a difference in the efficiency of transport of vigabatrin into the brain.

3.4.4 Vigabatrin kinetic inter-relationship between serum, CSF and ECF

Compared to serum, the CSF neuropharmacokinetic characteristics of vigabatrin are somewhat different. Firstly, mean T_{max} values in the CSF (0.8-1.2 hours) were larger than that in serum (0.4 hours). However, this would be expected since the difference represents the time needed for vigabatrin to pass from the blood compartment to the CSF compartment. Secondly, although there was a tendency towards equilibration of vigabatrin concentrations between the blood and CSF compartments this was not achieved by 8 hours after vigabatrin administration ((Figure 3.6). This is corroborated by the fact that there appears to be some significant scatter in vigabatrin concentrations when serum and CSF values are compared (Figure3.9).

Compared to serum, the ECF neuropharmacokinetic characteristics of vigabatrin are also different. Firstly, unlike the CSF compartment, equilibration between the ECF compartment (both frontal cortex and hippocampus) and the blood compartment was attained by approximately 7 hours after vigabatrin administration (Figures 3.7 and 3.8). This is corroborated by the fact that there is a significant correlation between ECF

and serum vigabatrin concentrations (figure 3.10). Secondly, and perhaps more importantly, the vigabatrin concentrations achieved in ECF were not only substantially lower than that seen in serum but additionally there was specific region distribution of vigabatrin. Thus vigabatrin concentrations in the frontal cortex were 2-fold greater than concentrations achieved in the hippocampus.

3.4.5 Summary

This is the first study to investigate the temporal pharmacokinetic inter-relationship of vigabatrin in rat blood, CSF and brain (frontal cortex and hippocampus) ECF.

1. Vigabatrin is associated with linear peripheral and central kinetics and with rapid penetration into the CSF and brain (frontal cortex and hippocampus) ECF compartment.

2. Although vigabatrin is not protein bound in serum, CSF concentrations are only 3-5% of those in serum and thus do not reflect free drug concentrations.

3. Vigabatrin was associated with differential brain region distribution in that vigabatrin concentrations in the frontal cortex were 2-fold greater than concentrations achieved in the hippocampus.

These data support an active uptake mechanism in the central nervous system. This would explain the means by which a highly water soluble drug passes into the central nervous system, and also the different concentrations attained in different brain areas.

Chapter 4

Pharmacokinetics and neuropharmacokinetics of levetiracetam

4.1 INTRODUCTION

Levetiracetam is a novel AED that not only possesses potent anticonvulsant properties but also exhibits antiepileptogenic effects (Gowers et al., 1992; Loscher et al., 1998). It exhibits a broad spectrum of action and is licensed for clinical use as adjunctive treatment for the management of patients with partial seizures (Perucca & Johannessen, 2003; Czapinski et al, 2005). The pharmacokinetics of levetiracetam in man have been extensively determined, and are simple and straight forward comprising of rapid absorption following oral ingestion resulting in peak plasma concentrations within 20 minutes and an elimination half-life of 6-8 hours (Patsalos, 2003, 2004). Such linear dose-dependent pharmacokinetic characteristics have recently been confirmed in a freely behaving rat model (Doheny et al., 1999). Levetiracetam was observed to rapidly and readily enter the CSF compartment, however, its efflux from CSF (mean $t_{1/2}$ range, 4.4-4.9 hours) was significantly slower than that suggested by serum concentrations (mean $t_{1/2}$ range, 1.8-2.8 hours). Since the brain neuropharmacokinetics of levetiracetam are unknown, the present study sought to investigate the serum and ECF (frontal cortex and hippocampus) kinetic inter-relationship of levetiracetam.

4.2 EXPERIMENTAL PROTOCOL

Male Sprague-Dawley rats (Charles River, Margate, Kent) weighing 300-350g were used. A venous catheter was surgically implanted into the right jugular vein and two microdialysis probes (one in the cerebral cortex and the other in the hippocampus) were stereotaxically implanted. The surgical procedures are described in Chapter 2. Two days after surgery, baseline samples were collected and the rats were then

administered levetiracetam (40 and 80mg/kg) by intrapeartoneal injection. Venous blood samples (100 µL) were withdrawn at 20 minute intervals for the first hour and at 30 minute intervals for a further 7 hours. Dialysate samples (20 µL) were collected at 20 minute intervals for 4 hours and then subsequently at 30 minute intervals for a further 4 hours. Levetiracetam concentrations were determined by HPLC using the method of Ratnaraj et al (1996) as described in Chapter 2.

4.3 RESULTS

4.3.1 Serum pharmacokinetics

The serum concentrations versus time profiles of levetiracetam after 40mg/kg and 80mg/kg levetiracetam administration are shows in Figure 4.1. Levetiracetam demonstrated rapid absorption and dose dependent kinetcis. Peak levetiracetam concentrations (T_{max}) were achieved at 0.4-0.7 ± 0.1 h (mean ± sem) and there was no significant effect of dose ($p > 0.05$), although there was a tendency for the concentrations to peak later at the higher dose.

Table 4.1 shows that apparent pharmacokinetic constants for individual rats together with the mean values, as calculated from the concentration versus time plots. The pharmacokinetic constants for individual rats showed some variability within each dose group. The mean C_{max} and AUC values for levetiracetam increased dose-independently and the values of C_{max} and AUC are more twice higher than that of 40mg. However, $t_{1/2}$ values were not dose dependent. The C_{max} and T_{max} values for levetiracetam were determined by visual inspection of the concentration versus time

curves and as the first sampling time point was at 20 minute, these values may have been overestimated or underestimated respectively.

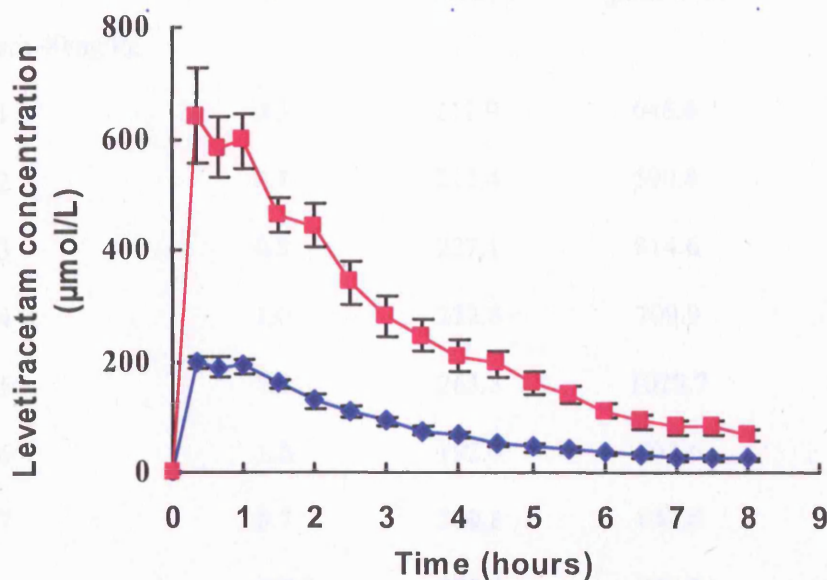


Figure 4.1: Serum levetiracetam concentration versus time profiles after intraperitoneal administration of 40 mg/kg (◆), and 80 mg/kg (■) levetiracetam. Values are mean ± sem of seven animals.

Table 4.1: Serum levetiracetam pharmacokinetic constants after intraperitoneal administration of 40 and 80 mg/kg levetiracetam

Rat No.	T_{max} (h)	C_{max} (μmol/L)	AUC (μmol /L/h)	$t_{1/2}$ (h)
Levetiracetam 40mg/kg				
1	0.3	211.9	648.6	2.3
2	0.7	212.4	590.8	1.9
3	0.3	227.1	814.6	2.3
4	1.0	222.8	709.9	2.1
5	1.0	263.3	1029.7	3.0
6	1.0	192.0	703.6	2.5
7	0.7	269.8	630.6	1.7
Mean	**0.7**	**228.5**	**732.5**	**2.2**
± s.e.m	**0.1**	**10.7**	**56.5**	**0.15**
Levetiracetam 80mg/kg				
1	0.3	709.6	2651.9	2.6
2	0.7	650.5	1874.2	2.1
3	0.3	705.3	2098.5	2.0
4	0.7	834.8	3507.8	2.5
5	0.3	902.9	2247.2	1.9
6	0.3	660.1	2182.9	2.3
7	0.3	432.7	1665.0	2.3
Mean	**0.4**	**699.4**	**2318.2**	**2.2**
± s.e.m	**0.1**	**56.7**	**230.0**	**0.1**

The free non-protein bound levetiracetam concentration was determined in 9 serum samples that spanned the concentration range and the time range studies in this study. Figure 4.2 shows the free/total levetiracetam concentration ratio for the nine samples and the calculated mean free fraction was 0.99 ± 0.06. These data suggest that levetiracetam is not protein bound.

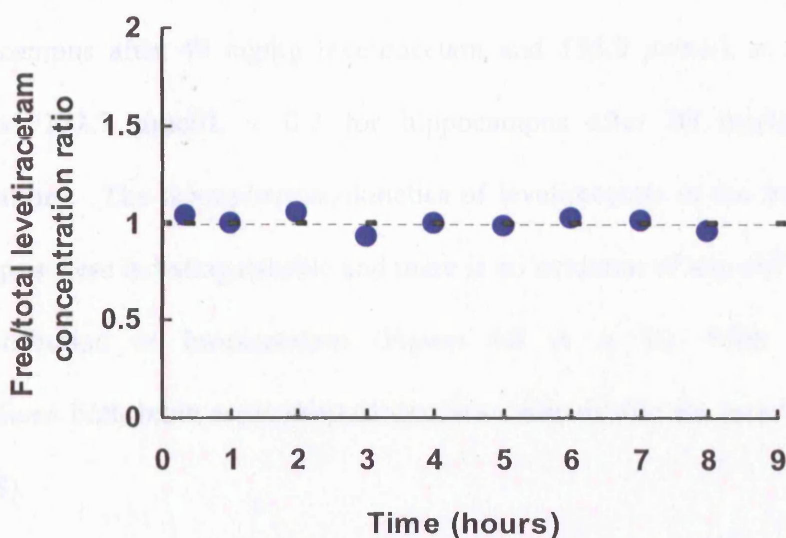


Figure 4.2: Levetiracetam free/total concentration ratios versus time profile.

4.3.2 ECF neuropharmacokinetics

The mean ± SEM *in vitro* relative recovery for levetiracetam from 24 microdialysis probes was 13.00 ± 0.69 % at a dialysate flow rate of 2 μL/min. These data were used to adjust the *in vivo* concentration data accordingly. Following 40 and 80 mg/kg levetiracetam administrated intraperitoneally, levetiracetam was rapidly detected in the dialysate of both the frontal cortex and the hippocampus (Figure 4.3 A & B).

Dialysate levetiracetam concentrations peaked at 1.8 hour (frontal cortex) and 2.5 hour (hippocampus) post levetiraceam administration and then declined exponentially. A similar profile was seen after 80mg/kg levetiracetam administration and, the levetiracetam concentration peaked at 2.1 hours (frontal cortex) and 1.9 hour (hippocampus). C_{max} values in the frontal cortex were similar to those observed in the hippocampus at both doses (69.5 μmol/L ± 8.7 for frontal cortex and 58.0 μmol/L ± 5.3 for hippocampus after 40 mg/kg levetiracetam and 155.0 μmol/L ± 21.4 for frontal cortex and 172.7 μmol/L ± 0.2 for hippocampus after 80 mg/kg levetiracetam administration). The neuropharmacokinetics of levetiracetam in the frontal cortex and hippocampus were indistinguishable and there is no evidence of any differential regional brain distribution of levetiracetam (Figure 4.4 A & B). With increased dose concentrations both brain areas showed the same time profile for levetiracetam (Figure 4.3 A & B).

The mean ± s.e.m neuropharmacokinetic constants as calculated from the log concentration versus time plots of individual rats are shown in Tables 4.2 and 4.3. Both AUC and C_{max} mean values increased dose-dependently and linearly. Levetiracetam was detectable at the time of first microdialysate sampling (15 min) with concentrations peaking some what later (mean $T_{max,}$ 2.0-2.5 hours) than serum (0.4-0.7hours). $T_{1/2}$ values were not significantly different when the two doses are compared.

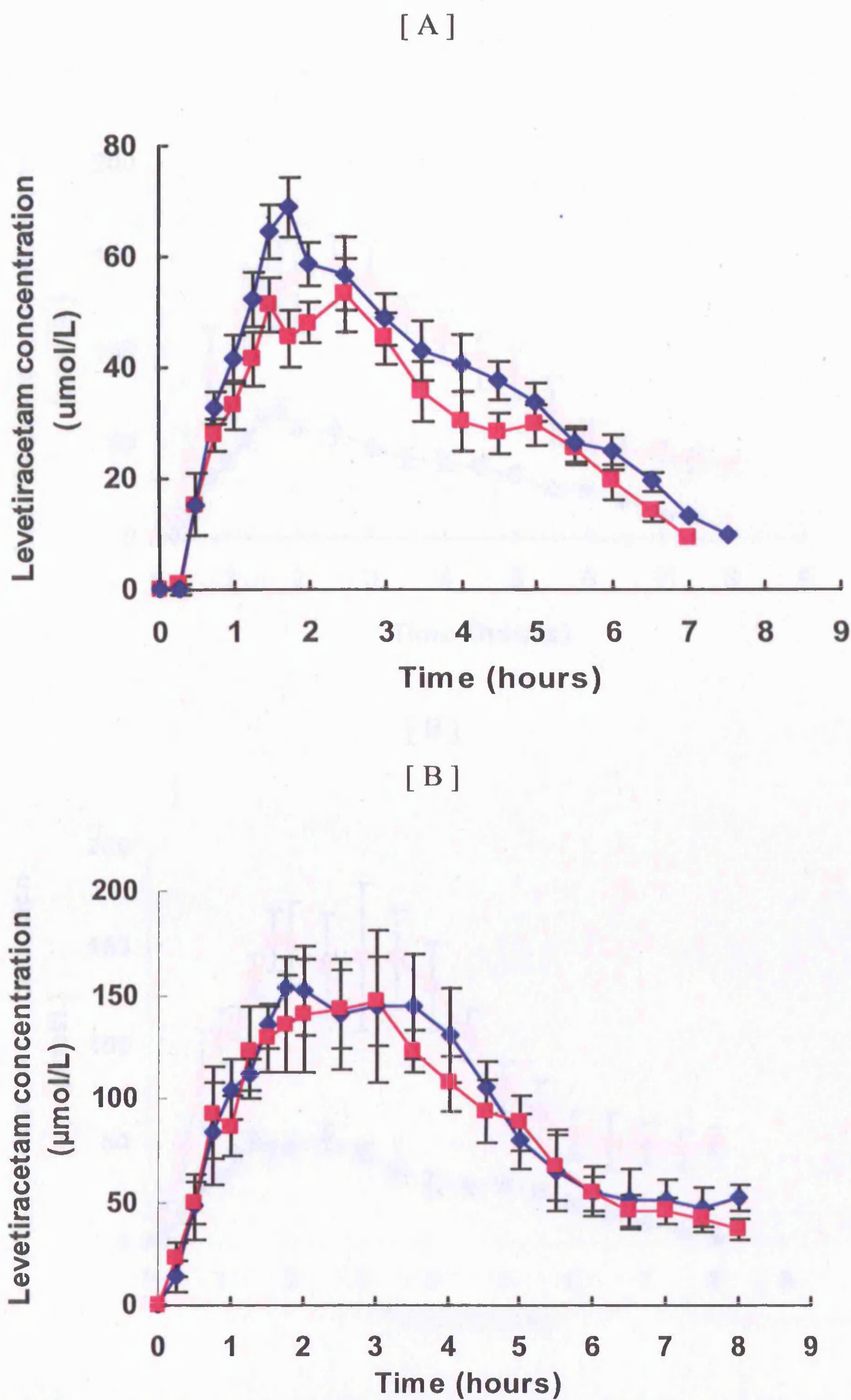
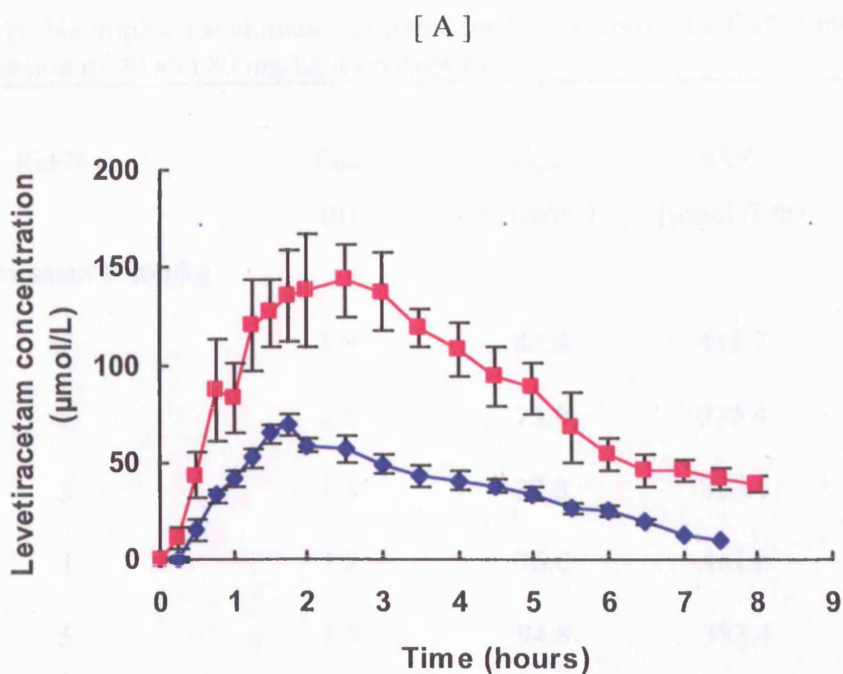


Figure 4.3: Levetiracetam ECF concentration versus time profiles for the frontal cortex (◆) and the hippocampus (■) after an intraperitoneal administration of levetiracetam (A) 40 mg/kg and (B) 80 mg/kg. Values are mean ± sem of 6 animals.

[A]



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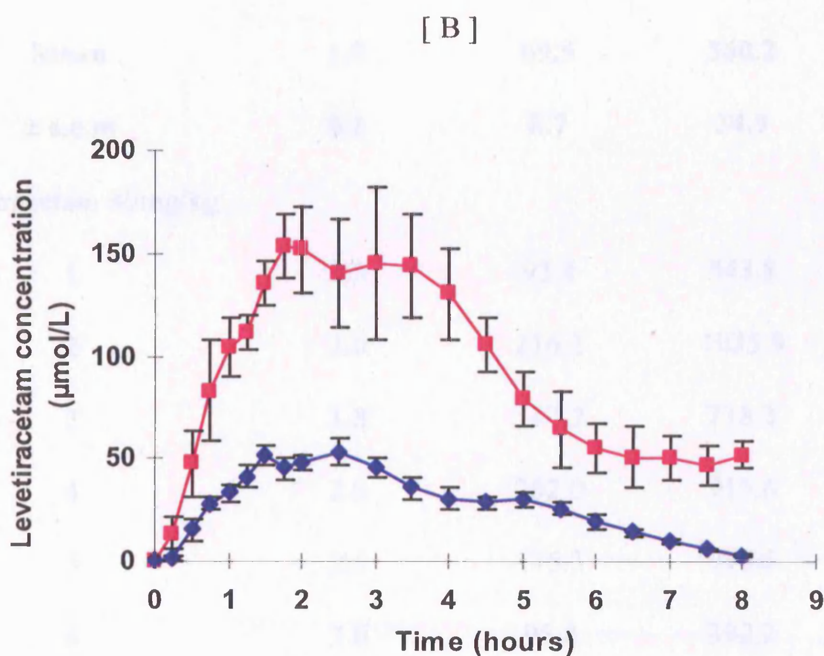


Figure 4.4: Levetiracetam ECF concentration versus time profiles for 40 mg/kg (◆) and 80 mg/kg (■) after an intraperitoneal administration of levetiracetam (A) in the frontal cortex and (B) in the hippocampus. Values are mean ± sem of 6 animals.

Table 4.2: Neuropharmacokinetic constants for frontal cortex ECF after intraperitoneal administration of 40 and 80 mg/kg levetiracetam

Rat No.	T_{max} (h)	C_{max} (μmol/L)	AUC (μmol /L/h)	$t_{1/2}$ (h)
Levetiracetam 40mg/kg				
1	1.8	82.8	415.7	3.5
2	2.5	75.0	375.4	2.9
3	1.8	37.3	335.1	2.8
4	1.8	76.0	461.8	3.3
5	1.5	94.8	383.4	2.7
6	1.3	51.3	189.6	3.1
Mean	**1.8**	**69.5**	**360.2**	**3.1**
± s.e.m	**0.2**	**8.7**	**34.9**	**0.1**
Levetiracetam 80mg/kg				
1	3.5	93.4	543.8	3.4
2	2.0	216.2	1035.9	3.1
3	1.8	147.2	718.3	3.3
4	2.5	202.0	913.6	3.1
5	2.5	175.3	940.5	3.3
6	3.0	95.8	392.2	2.9
Mean	**2.5**	**155.0**	**757.4**	**3.2**
± s.e.m	**0.3**	**21.4**	**86.7**	**0.1**

Table 4.3: Neuropharmacokinetic constants for hippocampal ECF after intraperitoneal administration of 40 and 80 mg/kg levetiracetam

Rat No.	T_{max} (h)	C_{max} (μmol/L)	AUC (μmol /L/h)	$t_{1/2}$ (h)
Levetiracetam 40mg/kg				
1	2.5	71.6	433.0	3.2
2	1.8	60.4	760.0	2.9
3	2.5	36.8	159.0	2.7
4	1.5	68.2	408.8	3.2
5	2.5	62.1	419.1	3.4
6	1.5	49.1	349.3	3.6
Mean	**2.1**	**58.0**	**421.5**	**3.2**
± s.e.m	**0.2**	**5.3**	**79.4**	**0.1**
Levetiracetam 80mg/kg				
1	2.5	235.0	1350.1	3.5
2	1.8	122.5	554.5	3.4
3	2.0	207.6	1159.4	3.7
4	2.0	168.7	723.6	3.3
5	2.0	175.0	758.3	2.9
6	1.3	127.3	480.8	2.8
Mean	**1.9**	**172.7**	**837.8**	**3.3**
± s.e.m	**0.2**	**18.0**	**140.6**	**0.1**

4.3.3 Levetiracetam kinetic inter-relationship between serum and ECF

After intraperitoneal administration levetiracetam demonstrated rapid absorption into both the serum and ECF compartments and the concentration of levetiracetam rose dose-dependently. The $t_{1/2}$ values of ECF were however longer than for serum. The AUC values and C_{max} values in the serum were three times higher than in the ECF (Figure: 4.5 and 4.6). However, compared to the levetiracetam $t_{1/2}$ in serum, the ECF $t_{1/2}$ values were significantly longer ($P < 0.05$).

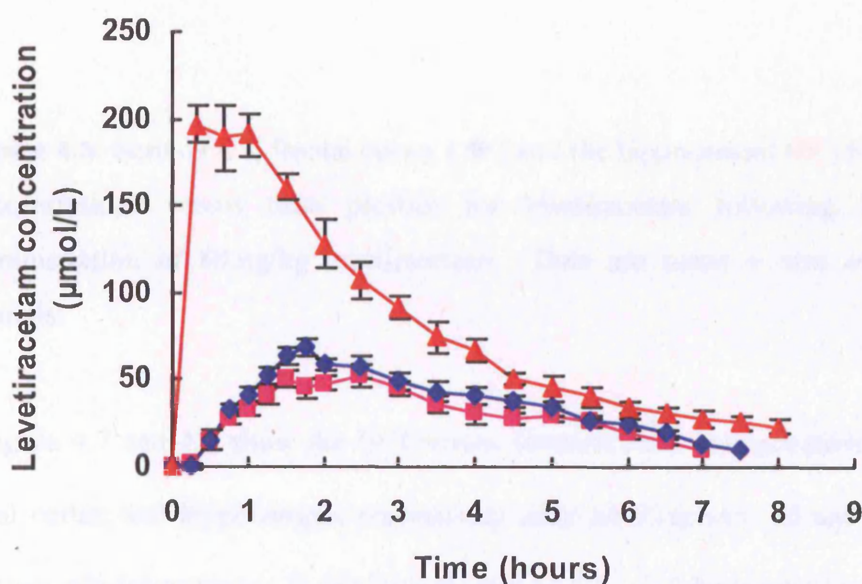


Figure 4.5: Serum (▲), frontal cortex (◆) and hippocampal (■) ECF concentration versus time profiles for levetiracetam following I.P. administration 40mg/kg levetiracetam. Date are mean ± sem of 6 animals.

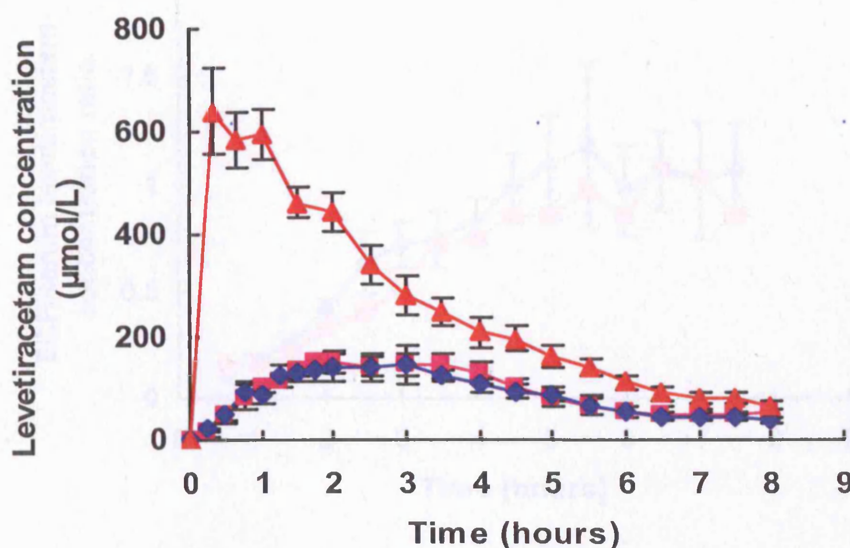


Figure 4.6: Serum (▲), frontal cortex (◆) and the hippocampal (■) ECF concentrations versus time profiles for levetiracetam following I.P. administration of 80mg/kg levetiracetam. Date are mean ± sem of 6 animals.

Figure 4.7 and 4.8 show the ECF/serum levetiracetam concentration ratios for the frontal cortex and hippocampus respectively after levetiracetam 40 and 80 mg/kg levetiracetam administrations. It can be seen that at 5.0 - 7.5 hour post levetiracetam administration, there is a tendency towards equilibration (as measured by a constant ECF/serum levetiracetam concentration ratios) between the blood and ECF compartments.

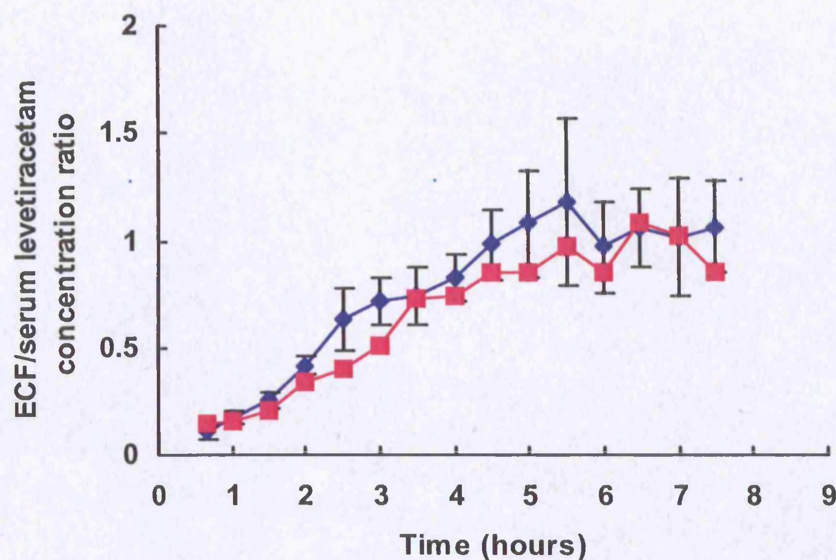


Figure 4.7: ECF/serum levetiracetam concentration ratio versus time profiles for the frontal cortex (◆) and the hippocampus (■) after 40mg/kg levetiracetam administration. Data are mean ± sem of 6 animals.

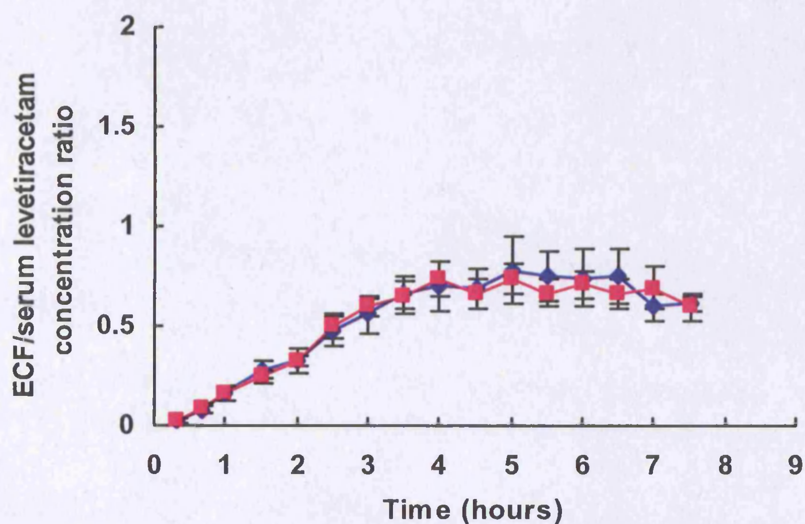


Figure 4.8: ECF/serum levetiracetam concentration ratio versus time profiles for the frontal cortex (◆) and the hippocampus (■) after 80 mg/kg levetiracetam administration. Data are mean ± sem of 6 animals.

4.4 DISCUSSION

Previously, Doheny et al (1999) reported on the temporal pharmacokinetic inter-relationship of levetiracetam in rat serum and CSF. In the present study, these data are extended to include the study of the temporal pharmacokinetic inter relationship of levetiracetam in rat serum and frontal cortex and the hippocampus ECF. The pharmacokinetic profile of levetiracetam in serum, as observed in the present study, was similar to that reported by Doheny et al (1999) and these data not only serve to further validate the animal model used, but also further confirm the predictable pharmacokinetic and neuropharmacokinetic nature of levetiracetam.

4.4.1 Serum pharmacokinetics

In this study, the temporal pharmacokinetics of levetiracetam in serum was determined after acute intraperitoneal administrations of 40 and 80mg/kg levetiracetam. Serum concentrations of levetiracetam rose quickly and dose-dependently. The mean C_{max} were 229 ± 10.7 μmol/L and 699 ± 56.7 μmol/L for 40 and 80 mg/kg levetiracetam respectively. T_{max} values were 0.4 – 0.7 hours, which suggests that levetiracetam is quickly absorbed from the peritoneal cavity into the systemic circulation. The serum elimination half-life of levetiracetam was 2.2 hours for both levetiracetam doses. Furthermore, levetiracetam was not protein bound in serum.

The pharmacokinetics of levetiracetam in the rat was previously reported by Doheny et al (1999). Levetiracetam were administered at three doses (20, 40 and 80 mg/kg) and the results showed that levetiracetam rapidly appeared in serum (mean T_{max}

0.25-0.5 hours) and these data are similar to those obtained in the present study (T_{max} 0.4-0.7). Similarly, the serum free/total concentration ratio (free fraction) reported by Doheny et al (1999) was 1.01 ± 0.02 (mean ± S.E.M.), which is almost identical to the present result of 0.99 ± 0.06 (mean ± S.E.M). Furthermore, the serum levetiracetam elimination half-life values observed by Doheny et al (1999) were 1.8 – 2.8 hours whilst in the present study a value of 2.2 hours was observed.

Following 250 to 5000 mg/kg levetiracetam oral administration to human healthy volunteers, levetiracetam was rapidly and almost completely absorbed. The absolute oral bioavailability was nearly 100%. Peak plasma concentrations (C_{max}) were achieved within 1 hour. The *V*d of levetiracetam was 0.5-0.7L/kg and was similar to total body water (0.6 L/kg) (Cereghino et al, 2000; Shorvon et al, 2002; Patsalos, 2000, 2003). In healthy young volunteers, the elimination half-lives of levetiracetam ranged from 6-8 hours. Levetiracetam was not bound (< 10%) to plasma proteins (Patsalos, 2000).

4.4.2 ECF neuropharmacokinetics

There are no published data describing the central neuropharmacokinetics of levetiracetam in the ECF. The present data are the first to report on the temporal ECF neuropharmacokinetics of levetiracetam in rat frontal cortex and hippocampus. These data reveal some interesting characteristics: 1.) The ECF concentration of levetiracetam rose linearly and dose-dependently 2.) ECF levetiracetam T_{max} values were longer compared to that observed in serum. 3.) The half-life values of levetiracetam in the ECF were longer than that in the serum; 4.) ECF levetiracetam concentrations in the

frontal cortex and the hippocampus were essentially identical and the distribution in the brain was not region special.

After intraperitoneal administration, levetiracetam rapidly appeared in both the frontal cortex and the hippocampus; levetiracetam was detected in ECF at time of first sample (0.25 hour). Peak levetiracetam concentrations in brain ECF occurred somewhat later (T_{max} were 1.8 – 2.5 hours) than in serum (T_{max} 0.4 –0.7 hours). These longer ECF T_{max} values represents the time for levetiracetam to pass from the serum compartment into to the brain ECF compartment (1.4- 1.8 hours). Furthermore, ECF T_{max} values were slightly longer than T_{max} values previously reported for CSF (T_{max} mean 1.6-1.9 hour) by Doheny et al (1999). The observed difference between the ECF and CSF compartments may be attributed, in part, to the methodology used, in that CSF was directly sampled whereas ECF was not, but may also represent differences between the ECF and CSF compartments.

ECF levetiracetam mean C_{max} values were 3.2 – 4.0 folds lower than those observed in serum and this difference may be attributed to the brain penetration characteristics of levetiracetam. However, a more likely explanation relates to the fact that ECF levetiracetam concentrations were calculated based on the *in vitro* recovery characteristics of microdialysis probe and is not dependent on Pgp or MRP transparters (Potschka et al, 2004). That ECF levetiracetam concentration increased dose-dependently and linearly would suggest that transport across the BBB was not rate limiting over the concentration range observed in the present study. This is further emphasised by the dose-dependent increase in ECF AUC values. Furthermore, as the

neuropharmacokinetic profiles of levetiracetam in the frontal cortex and hippocampus were essentially identical, it can be concluded that levetiracetam distribution in the brain is not region specific.

The efflux of levetiracetam from the brain ECF compartment appeared to be restricted, as demonstrated by the half-life of levetiracetam, which was approximately 50% longer than that observed in the blood. These data, combined with similar data for CSF (where efflux of levetiracetam from the CSF compartment was 170% slower. Doheny et al, 1999), may in part explain the substantial clinical efficacy of levetiracetam during twice a day dosing (Ben-Menachem & Falter, 2000; Cereghino et al, 2000; Shorvon et al, 2002), despite the fact that its blood pharmacokinetics (half-life 6-8 hour) would suggest that three times a day dosing is more appropriate.

Previously, it was reported that at equilibrium, the CSF/serum concentration ratio was similar to that of the blood free fraction (0.96 versus 1.01), suggesting the blood levetiracetam concentration reflected central brain concentrations (Doheny et al, 1999). However, in the present study, there was a tendency towards equilibration at 5.0 - 7.5 hours post levetiracetam administration and the ECF/serum concentration ratios for both the frontal cortex and the hippocampus ranged from approximately 0.7-0.8 (80mg/kg dose) to 0.9-1.0 (40mg/kg dose) at this time point. This would suggest that, in the acute situation, serum levetiracetam concentrations do reflect the concentrations at its site of action in the brain but that equilibration is achieved by 5 hours. However, it should be noted that since the ECF levetiracetam concentration data have been adjusted

on the basis of *in vitro* relative recovery and since *in vitro* recovery can be expected to be greater than *in vivo* recovery, ECF/serum concentration ratio may be overestimated.

4.4.3 Conclusions

1. Levetiracetam exhibits linear pharmacokinetics in serum and is not protein bound to serum proteins.

2. Levetiracetam rapidly and readily entered the brain ECF (frontal cortex and hippocampus) with mean T_{max} values of 1.8 – 2.5 hours.

3. The neuropharmacokinetic profiles of levetiracetam in the frontal cortex and hippocampus are essentially identical and levetiracetam distribution in the brain is not brain region specific.

Chapter 5

The effect of vigabatrin on CSF and ECF amino acids

5.1 INTRODUCTION

Excitatory and inhibitory amino acids, especially GABA and glutamate, have been proposed to play a critical role in the development and maintenance of epileptic seizures. GABAergic drugs have been developed in an effort to inhibit epileptic excitability and some of the new AEDs appear to directly or indirectly change brain GABA concentrations rather than act at a receptor or an ion channel (Czapinski et al, 2005). Vigabatrin is a drug that has a close structure to GABA but possesses a terminal vinyl group binding irreversibly to GABA-T, resulting in a blockage of metabolic degradation and an accumulation of GABA in neurones and glia (Bialer et al, 1999; Patsalos, 1999).

Vigabatrin affects on amino acid concentrations in CSF have been reported in many studies in both animal models and in patients (Ben-Menacham et al, 1988 & 1989; Ylinen et al, 1992; Riekkinen et al, 1989b; Halonen et al, 1990). However, the results are contradictory, but most studies show that CSF GABA, glycine, homocarnosine and taurine are increased, whilst glutamate and aspartate are reduced (Smolders et al, 1997; Jolkkonen et al, 1992; During et al, 1995; Richards et al, 1995; Schiffer et al, 2001). Furthermore, there are no studies that have investigated the inter-relationship between the neuropharmacokinetics of vigabatrin and concurrent changes in GABA and other amino acids. The present study sought to investigate these inter-relationships in a rat model, and to determine the effects of vigabatrin on CSF and ECF amino acids.

5. 2 EXPERIMENTAL PROTOCOL

Amino acid concentrations in CSF and ECF were measured using the methods described in Chapter 2. Male Sprague-Dawley rats (Charles River, Margate, Kent) weighing 300-350 g were used. CSF cannulae were implanted into the cisterna magna and microdialysis probes were inserted into the frontal cortex and the hippocampus as described in Chapter 2. Two days after surgery, baseline samples were collected and the rats were administered intraperitoneally with 500 and 1000 mg/kg vigabatrin for CSF and 1000mg/kg for microdialysis studies. CSF samples (10 μL) were collected every 30 minutes for 8 hours and dialysate samples (20 μl) were collected at 20 minute intervals for the first 4 hours and at 30 minute intervals for a further 4 hours.

5.3 RESULTS

5.3.1 CSF amino acid concentrations

Of the 16 amino acids that were measured in CSF and only the concentrations of 5 amino acids changed after vigabatrin administration. The results are shown in Table 5.1., 5.2 and Figures 5.1-5.5 show the data graphically. The other amino acid including alanine, asparagine, aspartate, glutamate, glutamine, isoleucine, phenylalanine, leucine, serine and threonine were unaffected by vigabatrin administration.

Table 5.1: Decreases of Arginine and Tyrosine concentrations in rat CSF expressed as a % of baseline after 500 and 1000 mg/kg vigabatrin administrations

Time	Arginine		Tyrosine	
(Hours)	500mg/kg	1000 mg/kg	500 mg/kg	1000 mg/kg
Baseline	100 ±10	100 ± 16	100 ± 9	100 ± 4
0.5	95* ± 11	102 ± 17	89* ± 7	86* ± 6
1.0	84* ± 10	99 ± 14	79* ± 4	69* ± 3
1.5	78* ± 7	85* ± 12	75* ± 3	63* ± 5
2.0	71* ± 8	86* ± 12	59* ± 2	56* ± 4
2.5	63* ± 7	84 ± 11	60* ± 5	58* ± 5
3.0	57* ± 6	67* ± 6	54* ± 7	49* ± 3
3.5	61* ± 4	68 ± 9	66* ± 6	54* ± 4
4.0	55* ± 6	61 ± 9	54* ± 5	58* ± 5
4.5	53* ± 5	65* ± 8	56* ± 2	49* ± 4
5.0	51* ± 6	60* ± 9	59* ± 6	47* ± 3
5.5	56* ± 5	60* ± 6	59* ± 4	47* ± 4
6.0	52* ± 6	56* ± 7	58* ± 3	49* ± 4
6.5	51* ± 6	52* ± 8	58* ± 6	51* ± 3
7.0	48* ± 6	55* ± 6	62* ± 5	47* ± 3
7.5	51* ± 6	54* ± 4	71* ± 6	50* ± 3
8.0	48* ± 5	48* ± 3	60* ± 4	47* ± 3

*

Data are mean of 5-6 animals

* = $P < 0.05$ compared to baseline

Table 5.2: Increases of glycine, homocarnosine and taurine concentrations in rat CSF expressed as a % of baseline after 500 or 1000 mg/kg vigabatrin administrations

Time	**Glycine**	**Homocarnosine**		**Taurine**	
(Hours)	**1000mg/kg**	**500mg/kg**	**1000mg/kg**	**500mg/kg**	**1000mg/kg**
Baseline	100 ± 11	100 ± 8	100 ± 10	100 ± 9	100 ± 4
0.5	108 ± 12	97 ± 7	104 ± 13	96 ± 6	91 ± 7
1.0	100 ± 9	98 ± 5	101 ± 13	85* ± 4	91 ± 7
1.5	122 ± 11	98 ± 5	108 ± 11	91 ± 7	102 ± 4
2.0	102 ± 10	99 ± 6	101 ± 13	94 ± 6	124 ± 8
2.5	100 ± 8	100 ± 10	121 ± 10	107 ± 6	136 ± 10
3.0	122 ± 10	92 ± 12	116 ± 9	103 ± 7	135 ± 11
3.5	140 ± 13	101 ± 8	120 ± 8	120* ± 8	153 ± 13
4.0	126 ± 12	110* ± 9	133 ± 10	126* ± 8	172*±14
4.5	132 ± 5	111* ± 10	132 ± 11	135* ± 8	172*±14
5.0	116 ± 2	112* ± 9	132 ± 10	131* ± 10	174*±13
5.5	136 ± 5	120* ± 110	149* ± 6	145* ± 8	175*±14
6.0	159* ± 7	120* ± 10	146* ± 13	163* ± 9	182*±15
6.5	158* ± 7	117* ± 11	150* ± 7	158* ± 9	191*±13
7.0	171* ± 11	125* ± 9	161* ± 13	170* ± 14	192*±15
7.5	151 ± 4	124* ± 9	159* ± 13	159* ± 10	199*±18
8.0	132 ± 9	125* ± 11	178* ± 14	138* ± 13	195*±11

Data are mean of 5-6 animals

* = $P < 0.05$ compared to baseline

Figure 5.1 shows CSF arginine concentrations over time after 500 and 1000 mg/kg vigabatrin administrations. Following 500mg/kg vigabatrin administration, arginine concentrations gradually decreased over time. At 8 hours (the last sample time point), the concentration of arginine was maximally reduced (48 % compared to baseline). Similarly, after 1000 mg/kg vigabatrin administration, arginine rose slightly at half an hour but subsequently declined to that which was observed after 500 mg/kg vigabatrin administration. The decline in arginine concentrations compared to baseline were statistically significant at both doses ($P < 0.05$).

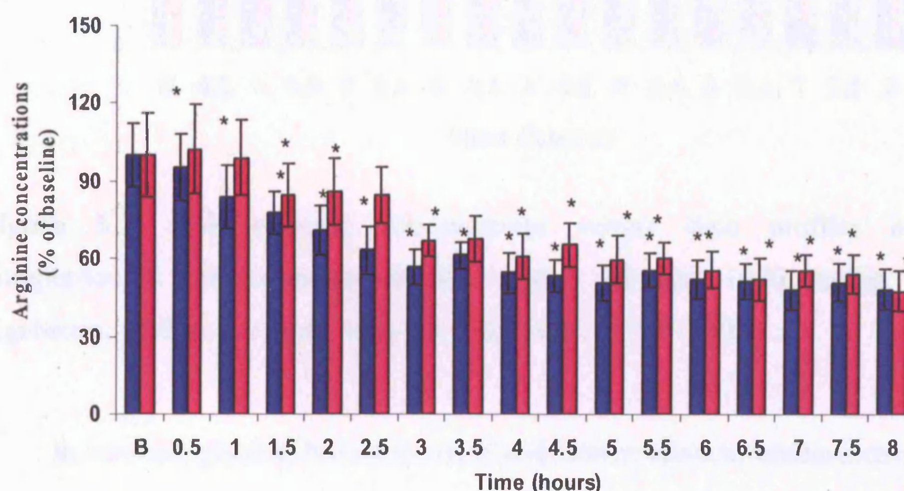


Figure 5.1: CSF arginine concentration versus time profiles after intraperitoneal administration of 500 mg/kg (■) and 1000 mg/kg (■) vigabatrin. Values are mean ± sem of 6 animals. * = $P< 0.05$

Figure 5.2 shows the temporal changes in tyrosine concentrations after 500 and 1000 mg/kg vigabatrin administrations. Tyrosine concentrations decreased at the first time point (0.5hour) and subsequently showed a time-dependent decrease. At 8 hours post vigabatrin administration, tyrosine concentrations were reduced by 40%

and 57%, after 500 and 1000 mg/kg vigabatrin respectively, when compared to baseline.

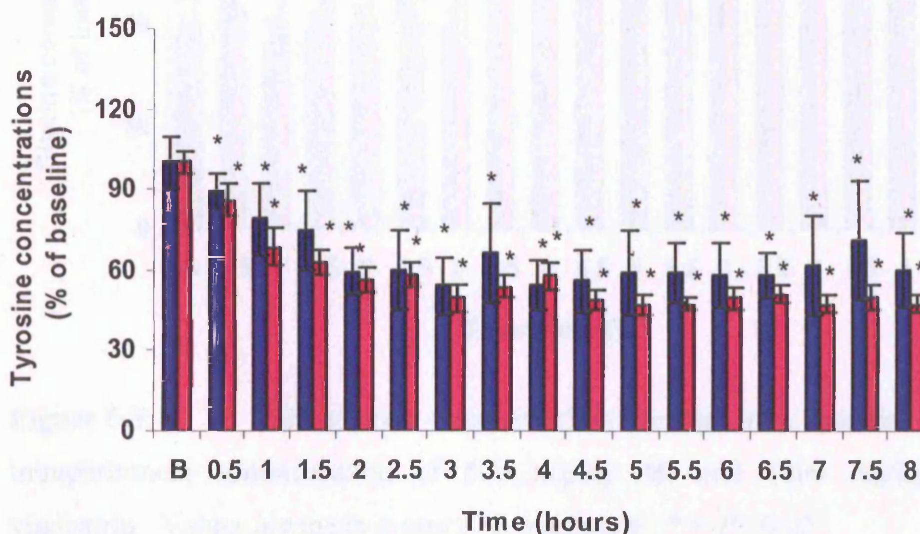


Figure 5.2. CSF tyrosine concentration versus time profiles after intraperitoneal administration of 500 mg/kg (■) and 1000 mg/kg (■) vigabatrin. Values are mean ± sem of 6 animals. * = $P < 0.05$

In contrast, glycine, homocarnosine and taurine concentrations increased after vigabatrin administration and these data are shown in Table 5.2 and Figures 5.3- 5.5. The elevation in glycine was only significant after 1000 mg/kg vigabatrin administration at 6-7 hours ($P < 0.05$) post dose. Concentrations were unaffected at other time points (Figure 5.3). Homocarnosine concentrations were elevated at 4 hours post vigabatrin administration and continued to rise until 8 hours (Figure 5.4). A similar pattern was observed for taurine (Figure 5.5). Thus at 7- 8 hours after vigabatrin 1000mg/kg administration, both homocarnosine and taurine were associated with an almost doubling of their concentration compared to baseline values.

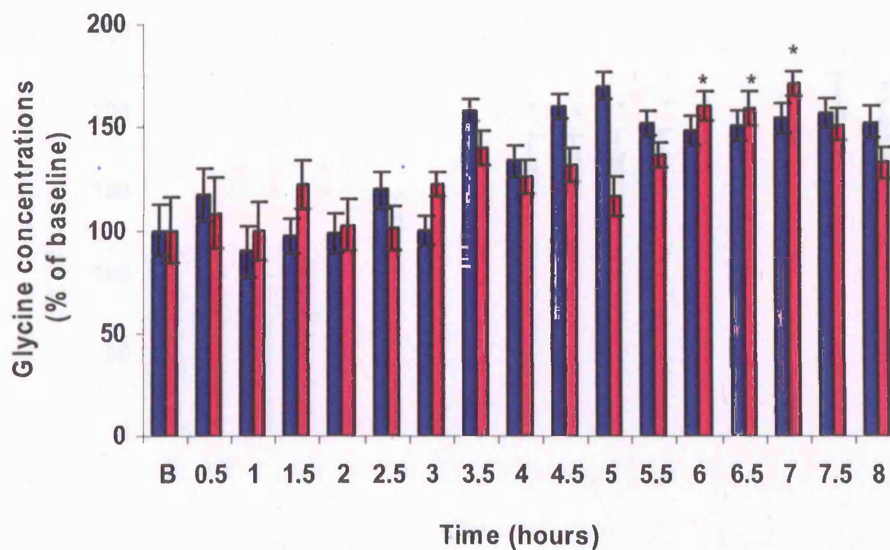


Figure 5.3: CSF glycine concentration versus time profiles after intraperitoneal administration of 500 mg/kg (■) and 1000 mg/kg (■) vigabatrin. Values are mean ± sem of 5-6 animals. * = $P < 0.05$

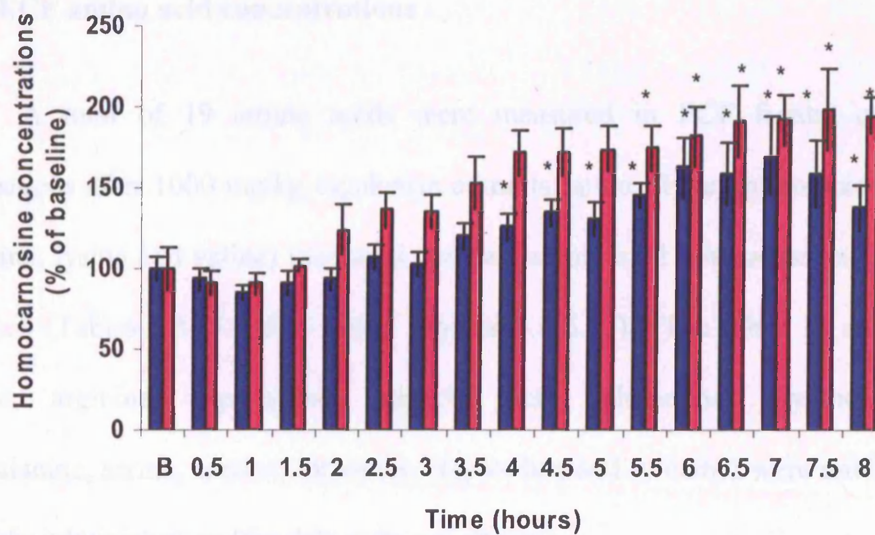


Figure 5.4: CSF homocarnosine concentration versus time profiles after intraperitoneal administration of 500mg/kg (■) and 1000 mg/kg (■) vigabatrin. Values are mean ± sem of 5-6 animals. * = $P < 0.05$

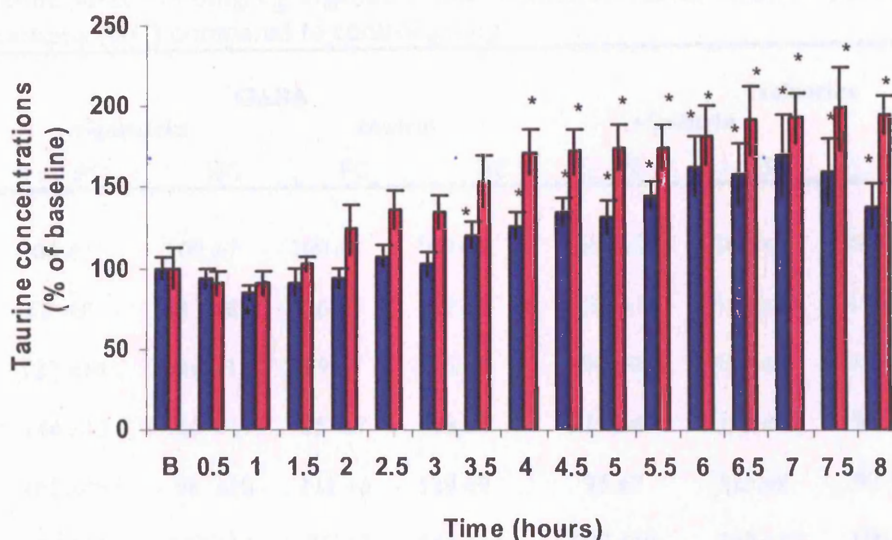


Figure 5.5: CSF taurine concentration versus time profiles after intra-peritoneal administration of 500 mg/kg (■) and 1000 mg/kg (■) vigabatrin. Values are mean ± sem of 5-6 animals. * = $P< 0.05$

5.3.2. ECF amino acid concentrations

A total of 19 amino acids were measured in ECF frontal cortex and hippocampus after 1000 mg/kg vigabatrin administration. Four amino acids (GABA, isoleucine, lysine and valine) increased and two amino aicd (glutamate and citrulline) decreased (Tables 5.3, 5.4 & 5.5 and Figures 5.6-5.17). The other 13 amino acids (alanine, arginine, asparagines, aspartic acid, glutamine, glycine, leucine, phenylalanine, serine, taurine, threonine, tryptophan and tyrosine) were unchanged by vigabatrin administration (the data were not shown).

Table 5.3: Increases of GABA and isoleucine concentrations in ECF expressed as a % of baseline after 1000mg/kg vigabatrin administration for the frontal cortex (FC) and hippocampus (HC) compared to control group

Time (h)	**GABA**				**Isoleucine**			
	vigabatrin		**control**		**vigabtrin**		**control**	
	FC	HC	FC	HC	FC	HC	FC	HC
0	100 ±7	100 ±7	100 ±3	100 ±4	100 ±7	100 ±3	100 ±5	100 ±4
0.3	67 ±8	117 ±8	136 ±5	107 ±5	110 ±11	148 ±13	108 ±7	102 ±9
0.7	123 ±10	110 ±11	119 ±7	116 ±7	96 ±8	120 ±11	93 ±6	88 ±8
1.0	144 ±13	86 ±11	85 ±7	118 ±7	89 ±6	122 ±10	87 ±7	85 ±9
1.3	161 ±16	99 ±10	111 ±6	129 ±9	95 ±7	113 ±9	92 ±8	91 ±7
1.7	137 ±15	113 ±14	85 ±7	144 ±11	109 ±10	169 ±10	100 ±9	116 ±12
2.0	119 ±16	97 ±12	104 ±8	120 ±13	97 ±11	131 ±14	91 ±6	85 ±10
2.3	127 ±17	76 ±6	99 ±7	107 ±10	111 ±8	147 ±16	99 ±9	111 ±11
2.7	205 ±19	109 ±9	99 ±6	110 ±11	113 ±6	133 ±16	92 ±9	100 ±13
3.0	162 ±13	87 ±8	104 ±9	101 ±12	98 ±13	132 ±14	78 ±6	106 ±12
3.3	209*±10	79 ±7	102 ±10	110 ±15	111 ±10	156 ±14	103 ±10	97 ±10
3.7	221*±16	109 ±14	107 ±10	130 ±11	116 ±11	153 ±12	98 ±11	102 ±11
4.0	203*±12	126 ±12	128 ±11	114 ±10	123 ±9	173 ±12	85 ±8	106 ±13
4.5	232*±17	97 ±7	115 ±9	103 ±9	125 ±13	161 ±11	83 ±7	117 ±9
5.0	281*±12	92 ±6	91 ±8	98 ±8	141 ±10	162 ±11	100 ±8	102 ±11
5.5	301*±16	106 ±9	153 ±10	112 ±9	121 ±12	176 ±16	107 ±10	104 ±6
6.0	334*±18	97 ±10	122 ±11	112 ±7	166 ±11	168 ±18	99 ±11	112 ±10
6.5	408*±23	102 ±13	146 ±14	107 ±7	170 ±17	183 ±25	108 ±12	107 ±11
7.0	442*±24	110 ±11	125 ±14	124 ±6	144 ±9	166 ±14	105 ±11	112 ±12
7.5	505*±29	142 ±13	137 ±13	108 ±10	174*±17	162 ±11	97 ±10	112 ±13
8.0	649*±18	140 ±14	123 ±12	123 ±11	191*±20	154 ±10	103 ±11	107 ±12

The data are mean data from 6 animals, * $P < 0.05$ compared to saline control.

Table 5.4: Increases of lycine and valine concentrations in ECF expressed as a % of baseline after 1000mg/kg vigabatrin administration for the frontal cortex (FC) and hippocampus (HC) compared to control group

Time (h)	Lysine				Valine			
	vigabatrin		control		vigabatrin		control	
	FC	HC	FC	HC	FC	HC	FC	HC
0	100 ±4	100 ±3	100 ±3	100 ±5	100 ±3	100 ±5	100 ±5	100 ±4
0.3	88 ±7	136 ±8	108 ±5	107 ±9	87 ±8	103 ±8	105 ±13	96 ±3
0.7	105 ±8	117 ±9	90 ±8	83 ±6	102 ±6	113 ±9	127 ±15	101 ±8
1.0	91 ±7	99 ±10	85 ±7	93 ±5	108 ±11	106 ±14	120 ±14	98 ±9
1.3	94 ±7	131 ±6	101 ±8	90 ±7	115 ±9	100 ±10	108 ±10	91 ±7
1.7	91 ±10	135 ±12	81 ±11	111 ±13	121 ±10	91 ±6	110 ±11	77 ±6
2.0	96 ±6	110 ±8	91 ±10	88 ±8	104 ±14	109 ±11	118 ±9	97 ±10
2.3	88 ±6	126 ±10	100 ±7	100 ±10	117 ±8	120 ±15	101 ±8	86 ±9
2.7	110 ±11	99 ±6	112 ±12	98 ±8	129 ±13	108 ±11	92 ±7	103 ±9
3.0	111 ±9	103 ±6	109 ±11	91 ±11	118*±8	109 ±7	96 ±7	98 ±8
3.3	109 ±10	136 ±10	90 ±8	93 ±10	140*±11	118 ±12	93 ±8	100 ±7
3.7	115*±11	141*±6	90 ±9	91 ±7	133*±10	106 ±8	96 ±9	98 ±6
4.0	113 ±11	133 ±13	90 ±7	94 ±6	142*±5	125 ±13	101 ±10	86 ±9
4.5	124*±11	137*±14	86 ±7	91 ±8	139*±6	130 ±9	104 ±10	95 ±7
5.0	124 ±15	128 ±12	101 ±10	113 ±9	147*±7	124 ±15	80 ±6	95 ±8
5.5	122 ±11	144 ±13	104 ±11	93 ±7	139*±9	126 ±8	79 ±5	94 ±7
6.0	144 ±10	110 ±13	100 ±8	89 ±8	166*±6	115 ±11	81 ±6	90 ±6
6.5	139 ±15	134 ±15	104 ±12	96 ±8	171*±10	115 ±12	87 ±7	85 ±7
7.0	130 ±12	115 ±12	107 ±13	88 ±7	135*±12	125 ±9	88 ±8	84 ±7
7.5	158 ±12	126 ±7	103 ±9	122 ±10	166 ±6	120 ±14	89 ±9	99 ±5
8.0	156 ±13	131 ±7	85 ±7	99 ±9	157 ±12	105 ±7	100 ±11	109 ±8

* This is the mean data (n = 6) from one way ANOVA $P < 0.05$

Figure 5.6 shows ECF GABA concentrations in the frontal cortex time profiles after 1000 mg/kg vigabatrin administration. It can be seen that GABA concentrations gradually increased over time. So that at 8 hours post-vigabatrin administration, GABA concentrations were 6.5 fold higher compared to that of baseline values ($P < 0.05$). In contrast, GABA concentrations in the hippocampus were unaffected by vigabatrin administration (Figure 5.7).

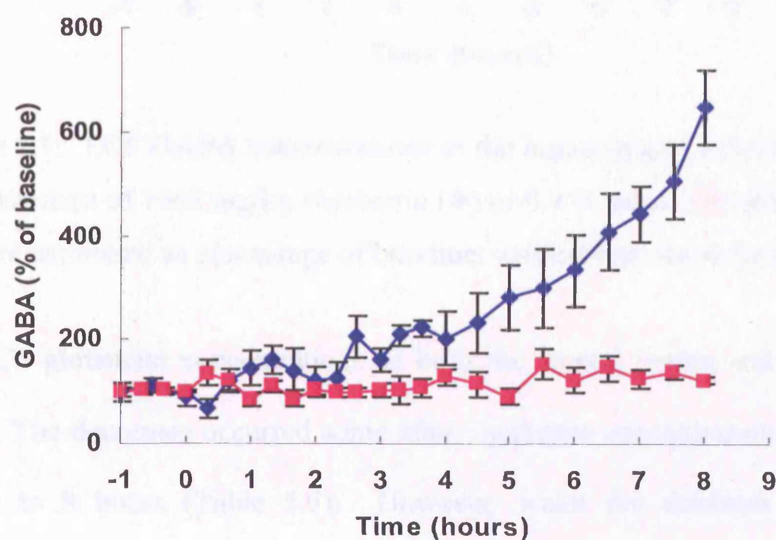


Figure 5.6: ECF GABA concentrations in the frontal cortex before and after administration of 1000 mg/kg vigabatrin (◆) or 0.9 % saline (■) of 6 animals. Data are expressed as percentage of baseline; vertical bars show s.e.m.

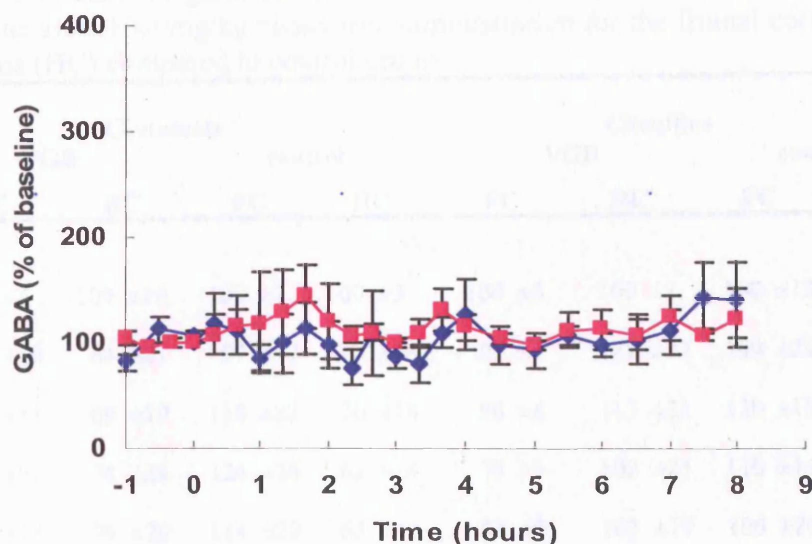


Figure 5.7: ECF GABA concentrations in the hippocampus before and after administration of 1000 mg/kg vigabatrin (◆) or 0.9 % saline (■) of 6 animals. Data are expressed as percentage of baseline; vertical bars show s.e.m.

ECF glutamate concentrations in both the frontal cortex and hippocampus decreased. The decreases occurred some after vigabatrin concentrations continued to decline up to 8 hours (Table 5.5). However, whist the decrease in glutamate concentrations attained statistical significance in the frontal cortex (Figure 5.8), the decrease was not statistically significant in the hippocampus (Figure 5.9). The latter observation can be attributed to the fact that saline (control) administered showed a concurrent decrease in hippocampal ECF glutamate concentrations.

Table 5.5: Decreases of glutamate and citrulline concentrations in ECF expressed as a % of baseline after 1000mg/kg vigabatrin administration for the frontal cortex (FC) and hippocampus (HC) compared to control group

Time (h)	Glutamate				Citrulline			
	VGB		control		VGB		control	
	FC	HC	FC	HC	FC	HC	FC	HC
0	100 ±2	100 ±16	100 ±2	100 ±3	100 ±3	100 ±2	100 ±13	100 ±4
0.3	66 ±10	84 ±21	97 ±17	73 ±16	95 ±7	122 ±27	109 ±22	104 ±21
0.7	67 ±11	69 ±10	118 ±23	70 ±14	96 ±6	113 ±21	120 ±19	144 ±25
1.0	63 ±8	74 ±24	126 ±26	62 ±14	78 ±9	102 ±21	110 ±16	97 ±11
1.3	62 ±13	74 ±20	114 ±28	63 ±11	89 ±9	103 ±19	106 ±24	83 ±13
1.7	66 ±12	62 ±23	125 ±27	63 ±11	89 ±7	112 ±22	124 ±24	72 ±21
2.0	51*±9	61 ±6	97 ±16	65 ±6	72 ±6	88 ±10	130 ±21	79 ±9
2.3	50*±9	54 ±18	109 ±26	57 ±9	75 ±8	98 ±13	117 ±26	116 ±26
2.7	61 ±13	51 ±9	100 ±21	64 ±9	95 ±18	77 ±8	77 ±16	108 ±7
3.0	44*±8	68 ±15	115 ±16	60 ±11	79 ±10	83 ±16	106 ±22	106 ±15
3.3	54*±18	70 ±16	118 ±21	61 ±8	77*±11	78 ±25	119 ±17	93 ±8
3.7	39*±10	56 ±11	130 ±27	58 ±6	74 ±7	86 ±22	129 ±20	96 ±7
4.0	52 ±13	76 ±23	115 ±13	62 ±10	81 ±8	79 ±15	107 ±20	71 ±13
4.5	51*±6	67 ±15	126 ±13	56 ±12	82 ±5	81 ±13	118 ±18	95 ±9
5.0	50 ±12	80 ±19	125 ±15	60 ±13	102 ±27	87 ±20	95 ±19	92 ±11
5.5	43*±7	71 ±16	118 ±18	66 ±13	71 ±6	72*±10	108 ±25	110 ±16
6.0	60 ±6	59 ±17	114 ±27	79 ±19	80 ±13	72 ±12	120 ±19	95 ±9
6.5	59 ±10	72 ±16	125 ±20	69 ±18	81 ±7	63 ±15	111 ±17	92 ±13
7.0	57 ±9	70 ±11	102 ±26	84 ±15	73 ±11	58*±13	113 ±22	111 ±13
7.5	54 ±5	63 ±8	124 ±27	73 ±12	74 ±9	69*±8	118 ±11	93 ±10
8.0	49 ±9	54 ±12	100 ±25	55 ±7	67*±7	64*±19	117 ±17	111 ±12

* This is the mean data (n = 6) from one way ANOVA, $P < 0.05$

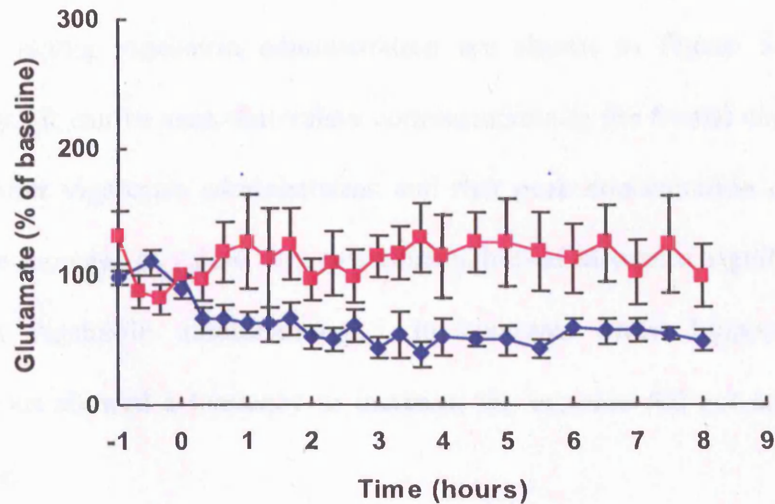


Figure 5.8: ECF glutamate concentrations in the frontal cortex before and after administration of 1000 mg/kg vigabatrin (◆) or 0.9 % saline (■) of 6 animals. Data are expressed as percentage of baseline; vertical bars show s.e.m.

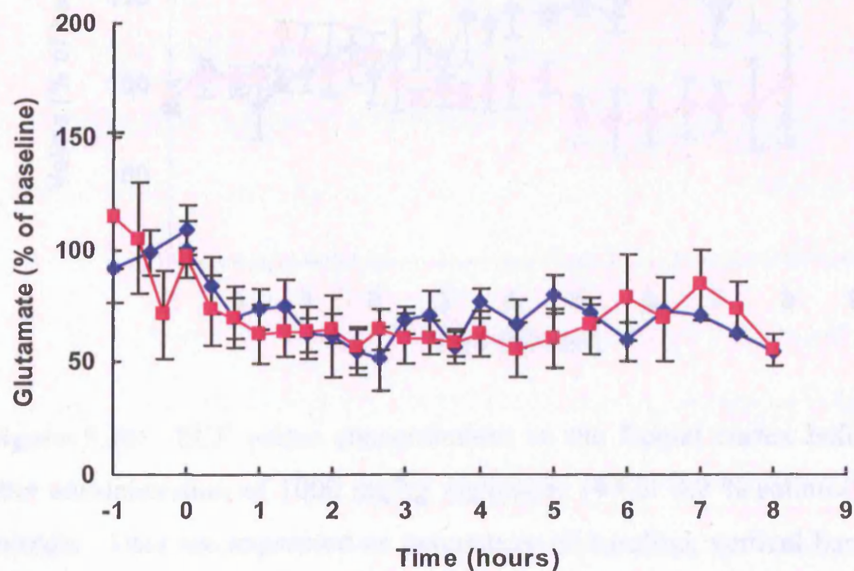


Figure 5.9: ECF glutamate concentrations in the hippocampus (◆) before and after administration of 1000 mg/kg vigabatrin or 0.9 % saline (■) of 6 animals. Data are expressed as percentage of baseline; vertical bars show s.e.m.

ECF valine concentration time profiles in the frontal cortex and hippocampus after 1000 mg/kg vigabatrin administration are shown in Figure 5.10 and 5.11 respectively. It can be seen that valine concentrations in the frontal cortex increased gradually after vigabatrin administration and that peak concentration occurred by 7 hours. The increase in valine concentrations achieved statistical significance by 3.0 hours post vigabatrin administration. In contrast, whilst hippocampal valine concentrations showed a tendency to increase, the increase did not attain statistical significance.

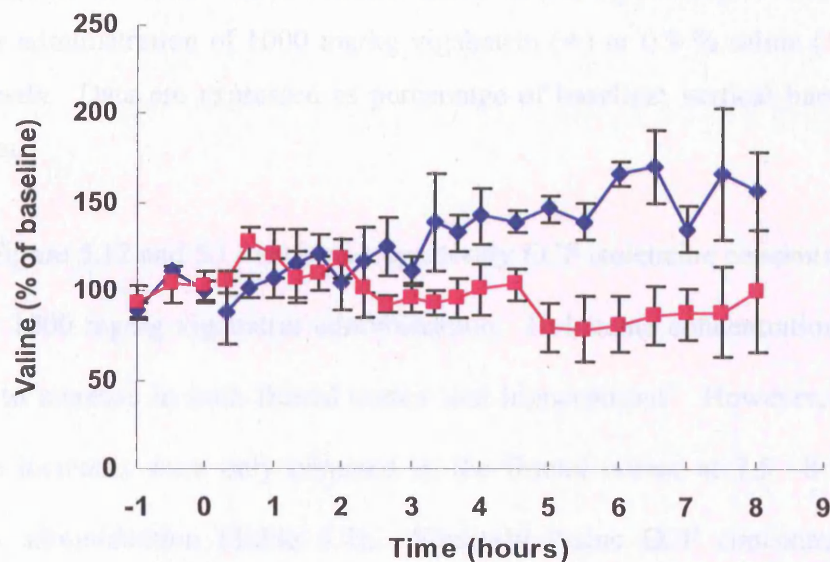


Figure 5.10: ECF valine concentrations in the frontal cortex before and after administration of 1000 mg/kg vigabatrin (◆) or 0.9 % saline (■) of 6 animals. Data are expressed as percentage of baseline; vertical bars show s.e.m.

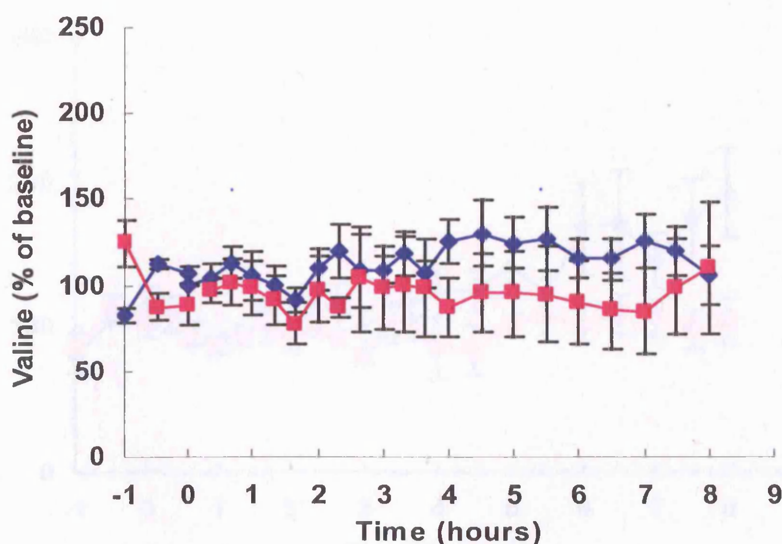


Figure 5.11: ECF valine concentrations in the hippocampus before and after administration of 1000 mg/kg vigabatrin (◆) or 0.9 % saline (■) of 6 animals. Data are expressed as percentage of baseline; vertical bars show s.e.m.

Figure 5.12 and 5.13 showed respectively ECF isoleucine concentrations over time after 1000 mg/kg vigabatrin administration. Isoleucine concentration showed a tendency to increase in both frontal cortex and hippocampus. However, significant isoleucine increases were only obtained in the frontal cortex at 7.5 –8 hours post vigabatrin administration (Table 5.3). Similarly lysine ECF concentrations also showed a tendency to increase in the frontal cortex and hippocampus (Figure 5.14 & 5.15). The increases in ECF lycine concentrations, however, only occurred between approximately 3.7 to 4.5 hours post vigabatrin administration ($P<0.05$). In contrast, ECF citrulline concentrations appeared to decrease in both the frontal cortex and hippocampus (Figure 5.16 and 5.17), but the changes were only statistically significant at 7-8 hours in both brain areas (Table 5.5).

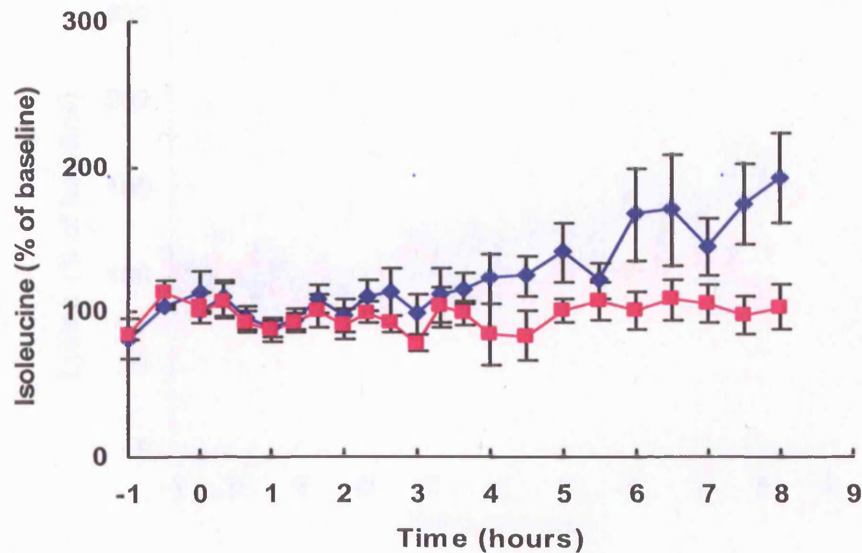


Figure 5.12: ECF isoleucine concentrations in the frontal cortex before and after administration of vigabatrin 1000 mg/kg vigabatrin (◆) or 0.9 % saline (■) of 6 animals. Data are expressed as percentage of baseline; vertical bars show s.e.m.

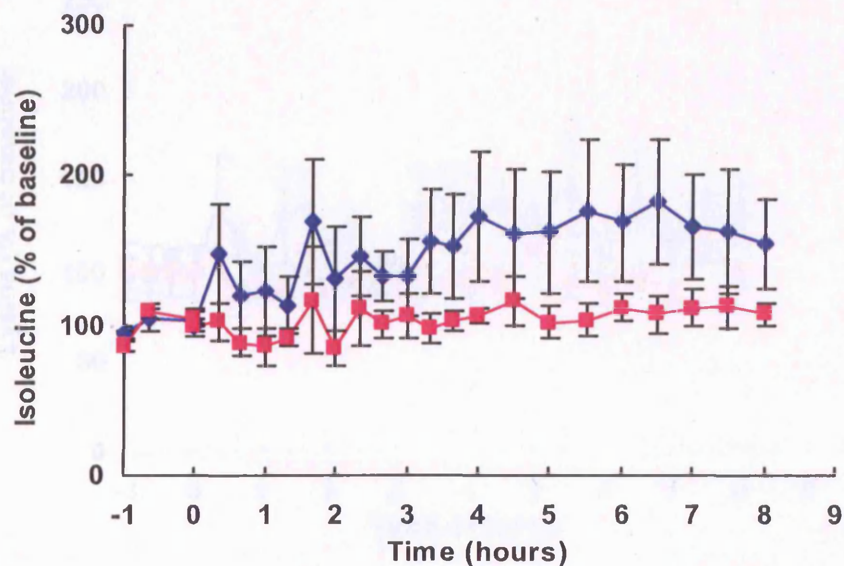


Figure 5.13: ECF isoleucine concentrations in the hippocampus before and after administration of vigabatrin 1000 mg/kg (◆) or 0.9 % saline (■) of 6 animals. Data are expressed as percentage of baseline; vertical bars show s.e.m.

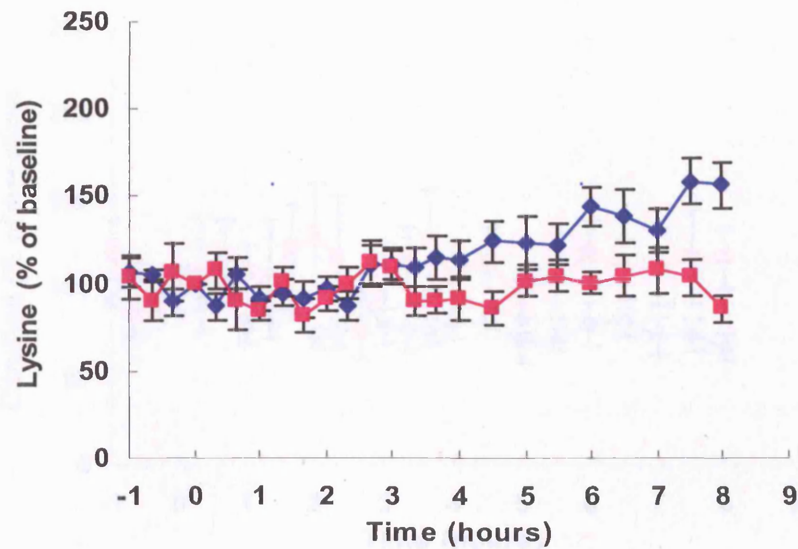


Figure 5.14: ECF lysine concentrations in the frontal cortex before and after administration of vigabatrin 1000 mg/kg vigabatrin (◆) or 0.9 % saline (■) of 6 animals. Data are expressed as percentage of baseline; vertical bars show s.e.m.

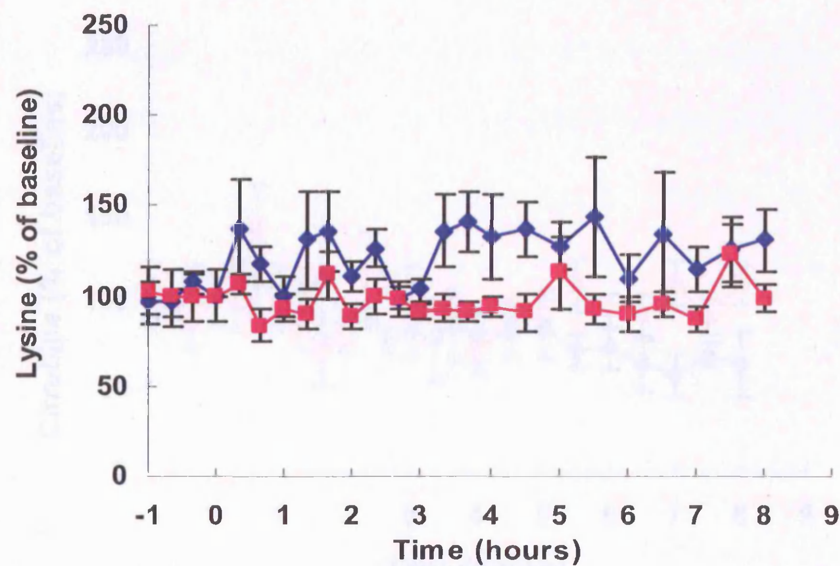


Figure 5.15: ECF lysine concentrations in the hippocampus before and after administration of 1000 mg/kg vigabatrin (◆) or 0.9 % saline (■) of 6 animals. Data are expressed as percentage of baseline; vertical bars show s.e.m.

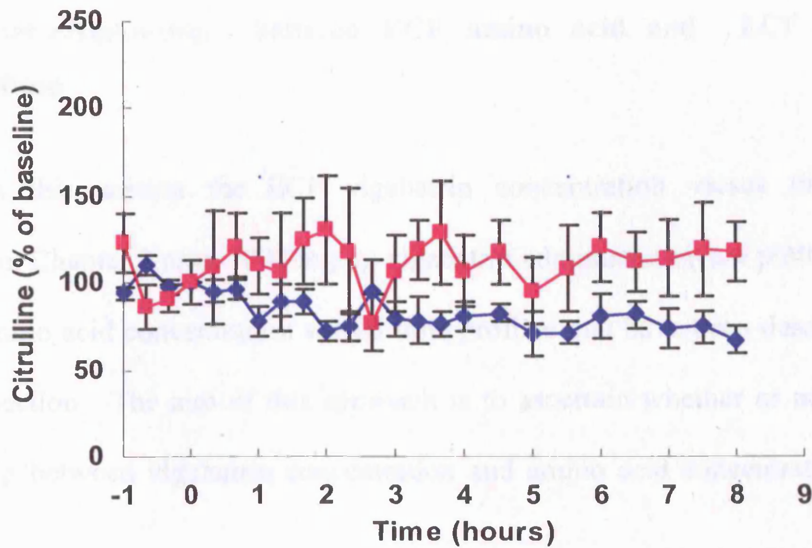


Figure 5.16: ECF citrulline concentrations in the frontal cortex before and after administration of 1000 mg/kg vigabatrin (◆) or 0.9 % saline (■) of 6 animals. Data are expressed as percentage of baseline; vertical bars show s.e.m.

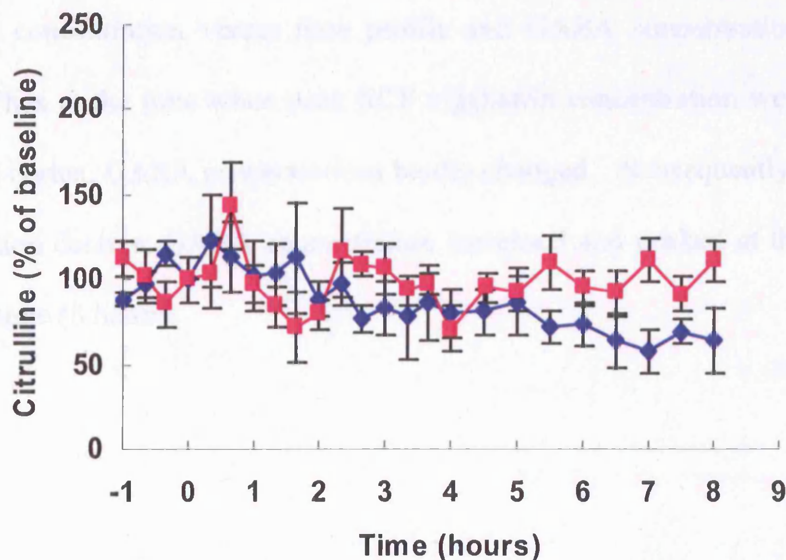


Figure 5.17: ECF citrulline concentrations in the hippocampus before and after administration of 1000 mg/kg vigabatrin (◆) or 0.9 % saline (■) of 6 animals. Data are expressed as percentage of baseline; vertical bars show s.e.m.

5.3.3 Inter-relationship between ECF amino acid and ECF vigabatrin concentrations

In this section the ECF vigabatrin concentration versus time profiles presented in Chapter 3 after 1000 mg/kg vigabatrin administration are plotted with that of ECF amino acid concentration versus time profiles that have been described in the previous section. The aim of this approach is to ascertain whether or not there is a relationship between vigabatrin concentration and amino acid concentration in brain ECF.

After 1000mg/kg vigabatrin administration, GABA concentrations rose gradually in the frontal cortex but not in the hippocampuas (Figure 5.18 and 5.19). Interestingly vigabatrin concentrations were also two-fold higher in the frontal cortex than that in the hippocampus. Furthermore, there was no correlation between the vigabatrin concentration versus time profile and GABA concentration versus time profile. Thus at the time when peak ECF vigabatrin concentration were achieved in the frontal cortex, GABA concentrations hardly changed. Subsequently, as vigabatrin concentration decline, GABA concentration increased and peaked at the time of last sampling time (8 hours).

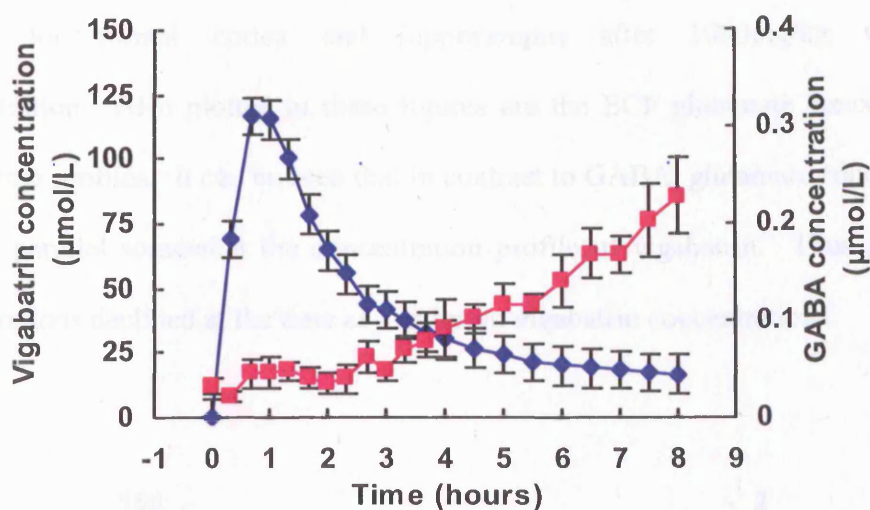


Figure 5.18: ECF concentration versus time profiles for vigabatrin (◆) and GABA (■) in the frontal cortex after 1000 mg/kg vigabatrin administration. Values are mean ± s.e.m of 6 animals.

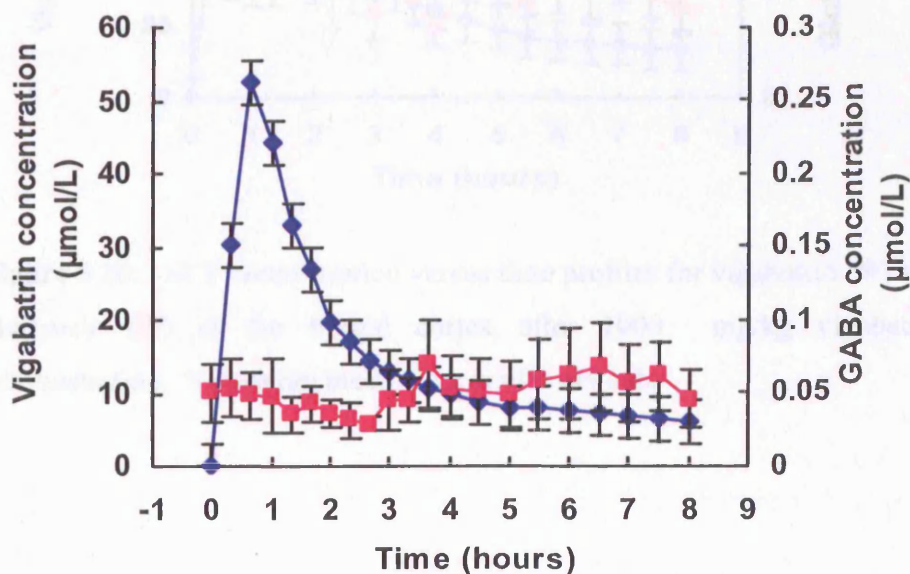


Figure 5.19: ECF concentration versus time profiles for vigabatrin (◆) and GABA (■) in the hippocampus after 1000 mg/kg vigabatrin administration. Values are mean ± s.e.m of 6 animals.

Figure 5.20 and 5.21 show respectively the ECF vigabatrin concentration profiles for frontal cortex and hippocampus after 1000mg/kg vigabatrin administration. Also plotted in these figures are the ECF glutamate concentrations versus time profiles. It can be seen that in contrast to GABA, glutamate concentration changes parallel somewhat the concentration profile of vigabatrin. Thus glutamate concentrations declined at the time as maximum vigabatrin concentrations.

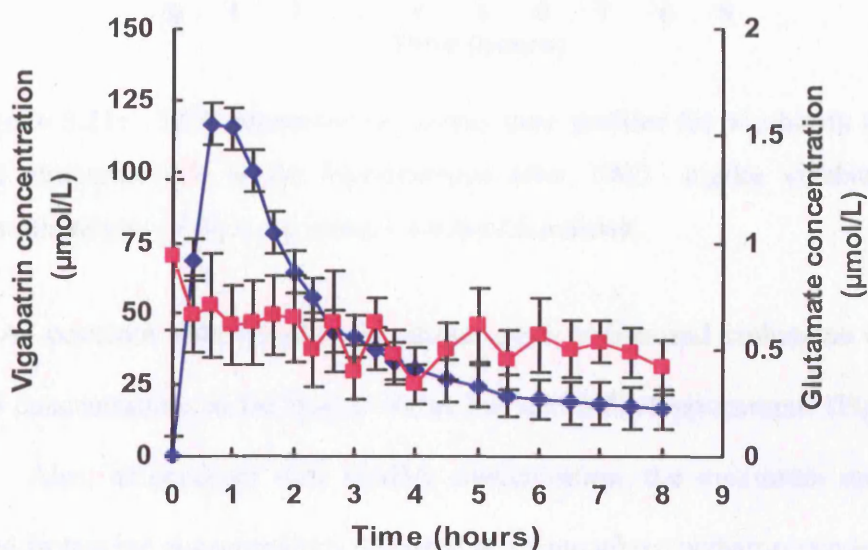


Figure 5.20: ECF concentration versus time profiles for vigabatrin (◆) and glutamate (■) in the frontal cortex after 1000 mg/kg vigabatrin administration. Values are mean ± s.e.m of 6 animals.

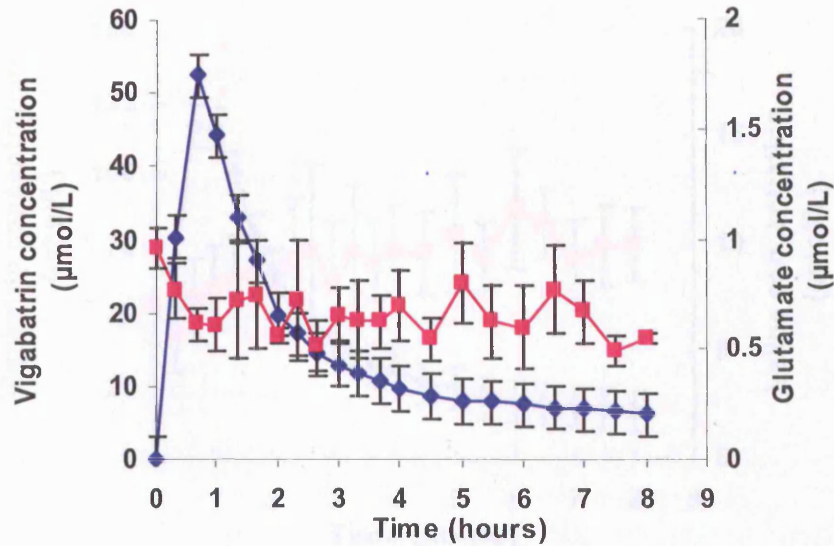


Figure 5.21: ECF concentration versus time profiles for vigabatrin (◆) and glutamate (■) in the hippocampus after 1000 mg/kg vigabatrin administration. Values are mean ± s.e.m of 6 animals.

As occurred with GABA, the amino acids valine and isoleucine exhibited increased concentrations in the frontal cortex but not in the hippocampus (Figure 5.22 & 5.23). Also, as occurred with GABA concentration, the maximum increase in valine and isoleucine concentrations occurred at a time after vigabatrin concentrations had peaked and indeed vigabatrin concentration were at their lowest level at the time of maximal amino acid concentration increases.

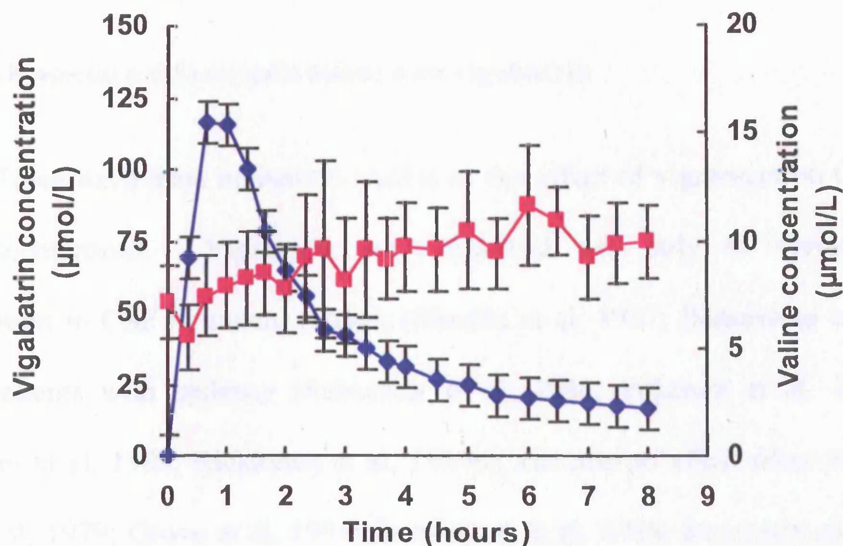


Figure 5.22: ECF concentration versus time profiles for vigabatrin (◆) and valine (■) in the hippocampus after 1000 mg/kg vigabatrin administration. Values are mean ± s.e.m of 6 animals.

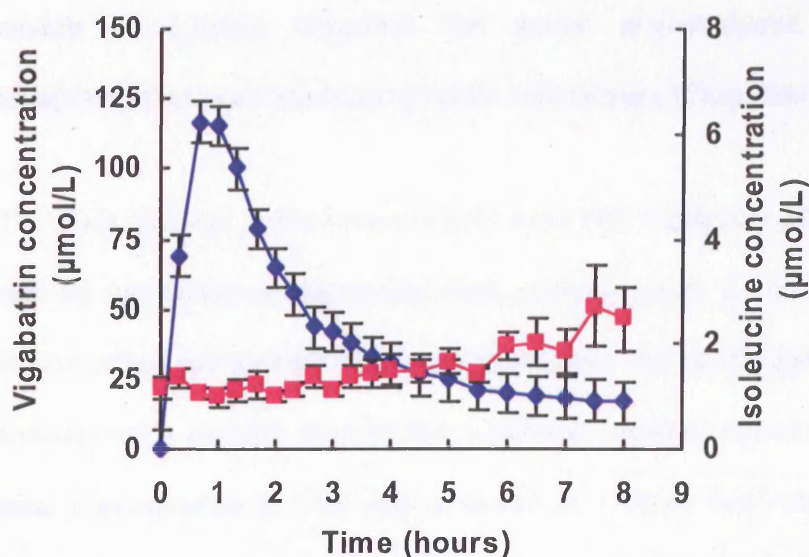


Figure 5.23: ECF concentration versus time profiles for vigabatrin (◆) and isolecine (■) in the frontal cortex after 1000 mg/kg vigabatrin administration. Values are mean ± s.e.m of 6 animals.

5.4 DISCUSSION

5.4.1 CSF amino acid concentrations and vigabatrin

There have been numerous studies of the effect of vigabatrin on CSF amino acid concentrations. Vigabatrin was observed not only to elevate GABA concentration in CSF in animal models (Piredda et al, 1987; Bernasconi et al, 1988) and in patients with epilepsy (Schechter et al, 1984; Pitkanen et al, 1988; Ben-Menachem et al, 1989; Riekkinen et al, 1989b), but also to affect other amino acids (Perry et al, 1979; Grove et al, 1981; Bernasconi et al, 1988; Ben-Menachem, 1989). These observations were of interest because 1. Many other amino acids, in addition to GABA, act as neurotransmitters and regulate normal brain function. 2. Some amino acids seem to have a role in the initiation, spread and suppression of seizures. 3. The neuropathological findings obtained from patients with chronic epilepsy or from animal models of epilepsy suggested that amino acid-mediated transmission (glutamate/aspartate) may participate in epileptic cell damage (Czapiriski et al, 2005).

The main findings in the present study were that vigabatrin affected several amino acids by increasing or decreasing their concentrations in rat CSF. These changes did not reflect nor parallel the concentration time course of vigabatrin in CSF. Neuropharmacokinetic analysis showed that vigabatrin rapidly crossed the BBB and that maximal concentration in CSF was achieved at 1 hour; however, amino acid concentration changes only occurred at 3-5 hours post vigabatrin administration. In the following section, each CSF amino acid change (in Tables 5.1 and 5.2) will be discussed. As the HPLC method was not sufficiently sensitive to measure the low concentrations of GABA in the CSF, GABA concentrations were not determined.

5.4.1.1 Homocarnosine

Homocarnosine, a dipeptide, is a major GABA conjugate found in patient CSF (Jansen et al, 2006). The interrelationships of homocarnosine and GABA metabolism are shown in Figure 5.24. The exact biologic function of homocarnosine in mammalian systems remains almost unknown. Homocarnosine may serve as a repository for GABA production (Jackson et al, 1994c). There is increasing evidence that homocarnosine has antiepileptic properties (Saransaari et al, 1999).

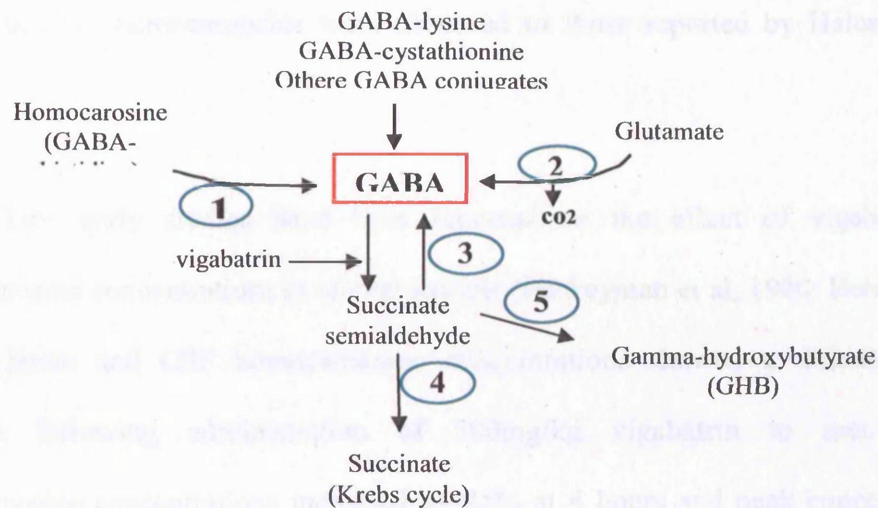


Figure 5.24: Interrelationships of homocarnosine and GABA metabolism. Numbered enzymes include: (1) carnosinase, (2) GAD, (3) GABA-T, (4) SSADH and (5) succinate semialdehyde reductase. Not all steps in this pathway are shown (Jansen et al, 2006).

In present study, CSF homocarnosine concentrations began to increase at approximately 2 hours after 500 and 1000 mg/kg vigabatrin administration. The increases were particularly apparent for the 1000 mg/kg vigabatrin dose, whereby homocarnosine concentrations significantly increased by up to 78% at 8 hours (Figure 5.4). After 500 mg/kg vigabatrin administration, CSF homocarnosine concentrations

were only increased by 25% at 8 hours post vigabatrin administration. Halonen and colleagues (1990) also reported the effect of vigabatrin on homocarnosine concentrations in rat CSF. CSF samples were collected at the 5, 24, 48 and 96 hours after 1000 mg/kg vigabatrin administration. Homocarnosine concentrations were increased by 56% and 123% at 5 and 24 hours respectively after vigabatrin administration. This latter concentration remained at the same level for the next 3 days. In the present study CSF samples were not collected at 24 hours post vigabatrin administration. However, the samples collected at 8 hours, suggest that comparable changes in CSF homocarnosine were observed to those reported by Halonen et al (1990).

Two early studies have been reported on the effect of vigabatrin on homocarnosine concentrations in animal models (Palfreyman et al, 1980; Bohlen et al, 1980). Brain and CSF homocarnosine concentrations showed a time-dependent elevation following administration of 500mg/kg vigabatrin to rats. CSF homocarnosine concentrations increased by 25% at 4 hours and peak concentrations occurred at 48 hours post vigabatrin administration (Palfreyman et al, 1980). Bohlen et al (1980) also found that vigabatrin elevated CSF homocarnosine concentrations in a dose-dependent manner. They administered vigabatrin at doses of 250, 500, 1000 and 2000 mg/kg and observed that CSF homocarnosine concentration increased linearly and dose dependently. The data presented in this thesis are consistent with these data.

There have been several reports of the effect of vigabatrin on CSF homocarnosine concentrations in patients with epilepsy (Ben-Menachem et al, 1989; Pitkanen et al, 1988; Kalviainen et al, 1993; Rothman et al, 1997; Petroff et al, 1998b

& 1999). Ben-Menachem (1989) used different vigabatrin dose intervals in 11 patients with drug-resistant complex partial seizures. CSF concentrations of total GABA and homocarnosine both increased dose dependently compared to baseline. Their results showed that daily dosing resulted in the highest concentration of CSF total GABA and homocarnosine compared to that of every second day dose, every third day and a single dose regimen. A similar study was reported by Halonen et al (1988) whereby patients with complex partial epilepsy were administered 3g vigabatrin per day for three months. In patients that experienced more than 50% reduction in seizures, free GABA, total GABA and homocarnosine concentrations in the CSF were increased by 104%, 151% and 194% respectively.

It is rather controversial whether or not homocarnosine free concentrations are a reflection or are correlated to GABA concentrations in the CSF and brain of animal models. An early study (Bohlen et al, 1980) has suggested that elevated CSF GABA concentrations primarily relate to altered GABA metabolism in the brain. Thus by measuring homecarnosine concentration it may be possible to monitor indirectly changes in brain or CSF GABA content during vigabatrin therapy. They also highlighted the fact that since homocarnosine occurred in relatively high concentrations in CSF, it can therefore be assayed more easily than free GABA which is present at very low concentrations in CSF. Recently Jansen et al (2006) have characterized the potential relationship between GABA and homocarnosine in patient CSF, they determined the total GABA levels in all control samples using gas chromatography and mass spectrometry. There was a highly significant linear correlation between total GABA and homocarnosine in control CSF. However, Palfreyman et al (1980) were not convinced that there is a good relationship between

CSF homocarnosine and GABA concentrations. Indeed their results showed that the correlation between the two parameters was not impressive (r = 0.64) and concluded that CSF homocanosine concentration is of only limited usefulness as an indicator of GABA concentrations in the CSF and brain.

It is not entirely clear as to how homocarnosine concentrations increase when GABA concentrations are also increased by vigabatrin. However, a likely explanation is that the enzyme, homocarnosine synthetase, might normally not be saturated with its substrate GABA (Palfreyman et al, 1980). Furthermore vigabatrin blocks normal GABA metabolism by inhibiting GABA-T; the increased intracellular GABA may also inhibit the metabolism of homocarnosine to GABA (Jansen et al, 2006). Overall, the increases in CSF homocarnosine concentration following vigabatrin administrations are likely to be related to altered GABA metabolism. Therefore monitoring of homocarnosine concentrations may provide a useful index of CSF GABA concentrations.

5.4.1.2 Glycine

In the present thesis, CSF glycine concentrations began to increase at approximately 1.5 hour after 1000 mg/kg vigabatrin administration. Thus compared to baseline concentrations, glycine concentrations were significantly increased (by 56 %) at 7 hours and the increase was somewhat lower (31%) at 8 hours. Although a similar pattern was observed after 500 mg/kg vigabatrin, the changes did not attain statistical significance. To date, only one other study has determined the effect of vigabatrin on glycine concentrations in rat CSF (Halonen et al, 1990). The data presented here

confirm the data presented by Halonen et al (1990). Their study entailed the administration of 1000mg/kg vigabatrin intraperiteally to freely moving rat. CSF samples were collected at the 5, 24, 48 and 96 hours post vigabatrin administration. CSF glycine concentration increased by 31% at 5 hours post vigabatrin administration, but concentrations failed to continually increase up to 24 hour.

In patients with epilepsy, glycine concentrations have been reported to increase consequent to chronic vigabatirn treatment. Halonen et al (1988) investigated 65 patients with complex partial epilepsy. CSF samples were collected before and after vigabatrin treatment for three months. Approximately 55% of patient showed a greater than 50% reduction in complex partial seizures. In these patient responders, CSF glycine concentrations increased significantly (P<0.001) compared with baseline values. In addition, however, CSF GABA and homocarnosine concentrations were also increased. Pitkanen et al (1988) using a group of patients with epilepsy, who were treated with vigabatrin for 7 months, reported that there was a 28% increase glycine in CSF compared to baseline values. Furthermore, CSF glycine concentrations in responders and non-responders patients were compared and it was observed that patients responding to treatment with vigabatrin had significantly higher CSF glycine concentrations (128%, compared to baseline values) than the non-responders.

That CSF glycine concentrations increase after vigabatrin administration in both the rat and patient with epilepsy may be contributable to two possible mechanisms. First, vigabatirn may enhance the release of glycine in the brain, where glycine may play a role as an inhibitory neurotransmitter. This possible mechanism

has been corroborated in part by the observation that CSF glycine concentrations are increased in patients with epilepsy that have good seizure control. The observation that the maximal protective activity occurred at 4 hours after vigabatrin administration to audiogenic sensitive rats whilst GABA-T was maximally inhibited 24 hours after the single dose of vigabatrin (Engelborghs et al, 1998) suggests that an increase in GABA may not be the sole mechanism of action of vigabatrin; alternative mechanisms may be via areduction in excitatory amino acid concentrations in the brain and/or elevation of glycine content (Czapinski et al, 2005). Second, glycinergic innervation is greatest at the level of the spinal cord (Ottersen & Storm-Mathisen, 1984). Thus, the enhancement of glycine concentration in CSF might be partly due to increased glycine release in the spinal cord consequent to vigabatrin treatment. The exact effect of vigabatrin treatment on the release of glycine and the role of increased glycine on the antiepileptic efficacy of vigabatrin treatment is not known and warrants further study.

5.4.1.3 Taurine

In the present study, CSF taurine concentrations were increased by 41% and 75 % at 5 hours after 500 and 1000 mg/kg of vigabatrin administration respectively (Table 5.2 and Figure 5.5). These observations are in agreement with another rat study in which CSF taurine concentration also increased after vigabatrin administration (Halonen et al 1990). Using a freely moving rat model, Halonen et al (1991) collected CSF samples at 5, 24, 48 and 96 hours after 1000 mg/kg vigabatrin administration. Taurine concentrations were observed to increase by 63% compared

to baseline at 5 hours post vigabatrin administration. However, this increased was not sustained at 24 hours.

Differing results have been observed in patient CSF studies of taurine. CSF taurine concentrations were shown either unchanged (Halonen et al, 1988; Pitkanen et al, 1988; Kalviainen et al, 1993) or reduced in different types of epilepsy after vigabatrin administration (Crawford & Chadwick, 1987; Honda, 1984) or without vigabatrin administration (Rainesalo et al, 2004).

Anti-epileptic action of taurine has been recognised for many years (Kontro et al, 1983). The precise role of taurine in synaptic transmission is uncertain but its antiepileptic effects have been confirmed in several models of experimental epilepsy and in short-term clinical studies (Fariello et al, 1985; Lombardini, 1992; van Gelder, 1992; Richards et al, 1995). Several clinical trials involving taurine supplementation in epileptic patients have been reported in various trials and variable success has been achieved (Timothy & Birdsall, 1998). The mechanism of action of taurine is probably via binding to $GABA_A$ or to glycine receptors (Gupta et al, 2005). Therefore the increase in taurine that I observed may have an antiepileptic action.

5.4.1.4 Arginine and tyrosine

In the present study, CSF arginine concentration was significantly decreased (48 %) at 8 hours post 500 and 1000mg/kg vigabatrin administrations. The significant reduction of CSF arginine observed in this study have not been described before with vigabatrin administrations in either animal or human. There are different views as to the role of arginine in epilepsy. Arginine has been reported to have an additive effect

on the anticonvulsant action of diazepam (Vanaja & Jayakumar, 2001) and there is some evidence to suggest that L-arginine might prevent seizure activity when administered at high doses (Noyan & Gulec, 2000). Arginine concentrations have been reported to be high in the brains of animal seizure models (Gardiner et al, 1993; De Sarro et al, 1993) and plasma arginine concentrations are decreased during AED treatment of patients with epilepsy (Rao et al, 1993 and Ko et al, 1993).

The exact function of tyrosine is not known, however, there are no reported data on CSF or brain tyrosine concentrations after vigabatrin administration in either epileptic models or in patients with epilepsy. The decreases in tyrosine were found in this study and from my knowledge, this is the first study to demonstrate a reduction in tyrosine concentrations by 60% and 47% at 8 hours after 500 and 1000mg/kg vigabatrin administrations respectively. The mechanism of this effect is not known. Some studies indicated that CSF tyrosine concentrations were decreased in children with refractory epilepsy after ketogenic diet (an established treatment for medically refractory pediatric epilepsy) treatment (Dahlin et al, 2005). It has been proposed that CSF tyrosine may be precursors of norepinephrine (NE), and NE was found to be required for the antiepileptic effect of the ketogenic diet (Szot et al, 2001). The role and mechanism of the change in tyrosine and arginine that I observed are unknown.

5.4.1.5 Glutamate and aspartate

There is a controversy as to the effect of vigabatrin on CSF glutamate and aspartate concentrations. Thus glutamate and aspartate concentrations have been reported to decrease (Halonen et al, 1991; Kalviainen et al, 1993), increase (Halonen

et al, 1990) or not to change (Pitkanen et al, 1988) after vigabatrin administrations in both animal models and in patients with epilepsy. In animal studies, the effects of inhibitory and excitatory amino acids were studied 12 days after intraperitoneal vigabatrin administration. The glutamate and aspartate concentrations were unchanged in CSF but were increased in brain tissue of hippocampus (Halonen et al, 1991). These results are similar to the results in this thesis as glutamate decreased only showed in brain dialysate of the frontal cortex and hippocampus, but not in CSF. The effects on CSF amino acid concentrations by chronic administration of vigabatrin to patients with complex partial epilepsy have been also studied (Halonen et al, 1988; Pitkanen et al, 1988). In these patients, whilst vigabatrin administration resulted in a dose-dependent increase in GABA concentrations, glutamate and aspartate concentrations were unaffected. Furthermore, when vigabatrin was presented as add-on treatment with lamotrigine to young patients with generalised refractory epilepsy, no changes in CSF glutamate and aspartate concentrations were observed (Eriksson & O'Connor, 1999). In the present studies, CSF glutamate and aspartate concentrations were unchanged and these data are in agreement with the results published by Halonen et al (1988,1990 and 1991), Ben-Menachem et al (1989), and Pitkanen et al (1988).

5.4.2 ECF amino acid concentrations and vigabatrin

An important observation in my thesis is that there are notable differences between CSF and ECF amino acid changes following vigabatrin administration. This emphasises that these are two separate compartments and that although changes in ECF concentrations of a neurotransmitter would be expected to lead to comparable changes in CSF concentrations, this does not always occur. CSF and ECF production

have different mechanisms and the diffusion of ECF into the CSF (and vice versa) is complex (not least because of the numerous amino acid transporters present in brain).

5.4.2.1 GABA

After vigabatrin administration, the total level of GABA concentration in the frontal cortex was elevated, which was consistent with the high vigabatrin concentration in this brain area. Vigabatrin concentration in the frontal cortex was more than two fold higher than that in the hippocampus and GABA concentration was 6 times higher at 8 hours in the frontal cortex compared to the hippocampus. There is definite relationship between GABA and vigabatrin concentrations in the brain. In the following paragraphs, discussion will focus on how GABA concentration changes relate to vigabatrin administration.

In the present study, vigabatrin administration (1000 mg/kg) was associated with a significant increase in ECF GABA concentrations in the frontal cortex but not in the hippocampus (Table 5.3). The increase of GABA concentrations in the frontal cortex ECF only began to be apparent at 3 hours post vigabatrin administration and GABA concentrations continued to rise so that peak concentrations occurred by 8 hours post vigabatrin administration. At this time GABA concentrations were more than 6-fold higher than that of baseline values. These data are in agreement with data from several other studies. Jung et al (1977) reported that brain GABA concentrations increased 5-fold by 3-4 hours after 1000 mg/kg vigabatrin administration. These elevated GABA concentrations were maintained for at least 24 hours, subsequently GABA concentration slowly declined up to 5 days. Sayin et al (1995) studied ECF

GABA concentration changes in substantial nigra pars reticulate following 1000mg/kg vigabatrin administration. GABA concentration increased significantly after 3 hour (200% of control) and peak concentration (450% of control) occurred by 24 hours and these elevated GABA concentrations persisted up to 72 hours. That ECF GABA concentrations increase 3 – 4 hours after vigabatrin administration would suggest that the inhibition of GABA-T by vigabatrin is not instantaneous. Inhibition of GABA-T after vigabatrin administration continues for 3 days (Jung et al, 1977). The latter effect can be explained on the basis that as GABA-T is irreversibly destroyed by vigabatrin and as GABA-T regeneration requires protein synthesis, this is time dependent (many days).

Interestingly, the time course of vigabatirn associated increases in ECF GABA concentrations after acute perfusion of vigabatrin directly into microdialysis probes is different. Thus hippocampal and striatum ECF GABA concentrations increased immediately after the perfusion of vigabatrin into the microdialysis probe (Jolkkonen et al,1992, Bentiurquia et al, 2004). These elevations of GABA concentration remained until the end of vigabatrin infusion and normalized rapidly thereafter. A longer-term elevation of GABA concentrations by inhibition of neuronal and glial GABA-T was not observed during the two hours following vigabatrin administration. The immediate increase in GABA concentrations may be explained by an acute block of GABA uptake and is not the consequence of GABA-T (Richerson & Wu, 2004). Thus the results of the present study are in agreement with that of Jung et al (1977) and Valdizan et al (1999), which suggest that vigabatrin would not be an appropriate drug to use in a clinical setting where stopping seizures rapidly is crucial.

At 8 hours after vigabatrin administration ECF GABA concentrations were approximately 5-fold higher in the frontal cortex than that of the hippocampus (Table 5.3). As to the brain region variability of these effects, the mechanism is consistent with the neuropharmacokinetics of vigabatrin in the frontal cortex and hippocampus. Figure 5.18 and 5.19 show the concentration versus time profiles for ECF GABA and vigabatrin in the frontal cortex and hippocampus respectively. It can be seen that vigabatrin AUC and C_{max} values in the frontal cortex were more than two fold higher than that of the hippocampus. Thus higher vigabatrin concentrations could be expected to have a greater inhibitory effect on GABA-T resulting in turn in higher GABA concentrations. This is the first description of the differential kinetics of vigabatrin in the brain and clearly the pharmacological effect of vigabatrin in elevating GABA concentrations is directly related to its central brain neuropharmacokinetics.

5.4.2.2 Glutamate

Vigabatrin administrations were associated with a significant reduction in frontal cortex ECF glutamate concentration (Figure 5.8). However, the effect was only statistically significant after 4.5 hours post vigabatrin administration. In contrast, although ECF glutamate declined in the hippocampus, the decline also occurred in the control group and therefore does not appear to be vigabatrin related. That ECF glutamate concentrations decrease in the frontal cortex after vigabatrin administration has been reported previously. In an acute study, 1200 mg/kg administration vigabatrin was associated with a decrease in ECF glutamate concentration in the frontal cortex of rats after 4 hours post vigabatrin administration (Loscher & Horstermann, 1994). In fact the ECF glutamate concentration versus time profile reported in that study was

very similar to that observed in the present study, but the differences is that glutamate concentration in their study was reduced by only 20%, which is less than that observed by me in the present study (40-50 %). In another study where rats were administered 30 mg/kg vigabatrin for 7 days, ECF glutamate concentrations in the hippocampus were also decreased (Smolders et al, 1997).

My results differ however from results obtained by direct perfusion of glutamate throught the microdialysis probe into the striatum (Benturquis et al, 2004). In this study, glutamate concentrations exhibited a 37% increase immediately after acute infusion Similar to GABA concentration, this increase of glutamate concentration remained elevated until the end of the vigabatrin infusion and return to baseline quickly afterward. The mechanism of this increase and the disparity with other studies remains unexplained.

The mechanisms by which vigabatrin reduces ECF glutamate concentrations in thisstudy are unknown. One possible explanation is that reduction of GABA transaminase activity prevents the conversion of alpha-ketoglutarate from the Kreb's cycle to glutamate, so decreasing the glutamate pool. The decrease in this excitatory transmitter would be expected to have an antiepileptic effect.

5.4.2.3 Valine, isoleucine and lysine

ECF valine, isoleucine and lysine concentrations after vigabatrin administration increased in the frontal cortex and the hippocampus, but changes in the frontal cortex were greater (Figure 5.10 to 5.15). The increases occurred at 4-8 hours after vigabatrin administration. There is only one other on the effect of vigabatrin on

these amino acids (Bernasconi et al, 1988) and interestingly vigabatrin was similarly observed to increase these amino acid concentrations in the frontal cortex.

Valine, leucine and isoleucine , the branched-chain amino acids (BCAAs) have been reported to reduce brain excitability by increasing the latency to convulsive seizures induced by picrotoxin and pentylenetetrazol (PTZ) (Dufour et al, 1999), and also play an important role in brain neurotransmitter metabolism (Brosnan & Brosnan, 2006). It appears that neurological dysfunction can occur when BCAAs are too low or too high. How high or low levels of BCAAs can cause neurological dysfunction is not know (Harris et al, 2005). In the present study, although these three amino acids all increased in ECF of both brain areas, only valine and isoleucine changes were statistically significant. ECF valine concentrations in the frontal cortex were increased by 20-70 % from 3- 7 hours after vigabatrin administration. The observed increases are similar to those observed in studies of animal and patients with ischaemic brain damage (Kuchiwaki et al, 1994; Shimada et al, 1993; Karkela et al, 1992). In contrast CSF valine concentrations after vigabatrin administration were unaffected. The contribution of valine to the mechanism of action of vigabatrin, is presently unknown.

Isoleucine is another amino acid for BCAAs and previously acts as nitrogen donors in the cerebral glutamate-glutamine cycle. Approximately one third of glutamate is derived from branched-chain amino acids. In the present study, ECF isoleucine concentration increase by 10-90% in the frontal cortex and the hippocampus after 3 to 8 hours after vigabatrin administration. However, the changes were only statistically significant at 7.5- 8 hours. Hippocampus ECF concentration changes were

less substantial with concentration increases of 30-50% for isoleucine; these changes were not statistically significant.

As to how vigabatrin affect these two amino acids is unclear, but valine and isoleucine may have a role in reducing brain excitability (Dufour et al, 1999). Using PTZ, Dufour et al (1999) observed that 300 mg/kg leucine and isoleucine increased the latency to absence-like and tonic-clonic seizures but did not influence the duration of tonic-clonic seizures. A further study (Dufour et al, 2001) showed that BCAAs decreased brain glutamate concentrations in the thalamus and cortex without affecting GABA concentrations. Thus, BCAAs, by decreasing glutamatergic neurotransmission could decrease neuronal excitability (Dufour et al, 2001). Overall, therefore, the increase in ECF BCAAs as observed in the present study along with the data of Dufour et al (1999 and 2001) would suggest that valine and isoleucine, and possibly leucine may decrease ECF glutamate concentrations. This mechanism may contribute to anticonvulsant action of vigabatrin.

ECF lysine concentrations in both frontal cortex and hippocampus also appeared to increase after vigabatrin administration (Figures 5.146 and 5.15). A 10-50% increase was observed at 3-8 hours post vigabatrin administration. There is very little information as to the role of lysine in epilepsy. Bernasconi et al (1988) have reported a time- and dose-dependent elevation in lysine concentrations in cortical tissue. However, the increase in lysine concentration was less pronounced compared to the other amino acids studies. The mechanism as to how vigabatrin may increase lycine concentrations is not known.

5.4.2.3 Citrulline

There was a tendency for ECF citrulline concentrations, both in the frontal cortex and hippocampus to decrease after vigabatrin administration (Figures 5.16 and 5.17). These decreases were observed at 3 to 8 hours but were statistically significant only at 3.3 –3.7 hours in the frontal cortex. There are no published data on the effect of vigabatrin on ECF citrulline concentrations. The mechanism of this effect is unknown. Nevertheless, it has been shown that citrulline can suppress seizures in models of epilepsy (Talavera et al, 1997; Paul, 2002); a reduction in nitric-citrulline formation may be the mechanism by which this is mediated (Smith et al, 1996). In an animal seizure model, an increase in citrulline concentration was also seen with elevated glutamate and glycine concentrations (Richards et al, 2000). Whether the decrease in ECF citrulline concentrations is related to the mechanism of anticonvulsant action of vigabatrin needs further study.

5.5 CONCLUSIONS

1. In the CSF compartment, vigabatrin administration was associated with reduction in arginine and tyrosine concentrations and increases in homocarnosine, glycine and taurine concentrations. Other amino acids were not affected. GABA concentrations were not measured in CSF.

2. In the frontal cortex and hippocampus, ECF compartment, vigabatrin administration was associated with significant changes in a variety of amino acids (GABA, glutamate, isoleucine, lysine, citrulline and valine). However, not all amino acid concentration changes were statistically significant.

3. The vigabatrin associated amino acid changes in the CSF differ somewhat from those observed in ECF, emphasising that CSF and ECF represent distinct compartments.

4. Whilst ECF GABA concentration in the frontal cortex increased after vigabatrin administration, ECF GABA concentration was unaffected in the hippocampus. These changes can be explained on the basis that vigabatrin concentrations in the frontal cortex are 2-fold higher than those achieved in the hippocampus. The absence of changes in extracellular GABA detected by microdialysis in the hippocampus does not exclude that there may be local changes or changes in specific subregions of the hippocampus.

5. The observed increase in the frontal cortex ECF GABA concentrations, however, did not parallel the concentration versus time profile of ECF vigabatrin. Instead ECF GABA concentration increased as ECF vigabatrin concentration declined. This indicate that the change in GABA is not due to a direct effect of vigabatrin and is consistent with the known mechanism of action (GABA transaminase inhibition).

Chapter 6

The effect of levetiracetam on ECF amino acids

6.1 INTRODUCTION

Studies aimed at elucidating whether levetiracetam exerts its antiepileptic effects by an interaction with GABAergic mechanisms have led to somewhat contradictory results. Levetiracetam failed to interact with classical receptor and ion channel sites in the brain (Noyer et al, 1995). The drug has, however, been proposed to bind in a stereoselective manner to a specific site in CNS membranes where it was weakly displaced by several other AEDs and GABA-related substances (Noyer et al, 1995).

Loscher et al (1996) reported that levetiracetam induces changes in GABA metabolism and turnover in discrete areas of rat brain. Their results showed that systemic levetiracetam administration resulted in significant alterations in GAD and GABA-T activities in several brain regions, but these enzyme alterations were not the consequence of direct drug effects but rather a consequence of postsynaptic changes in either GABAergic or other amino acid related systems. Sills et al (1997) also studied several GABA-related neurochemical parameters in mouse brain after levetiracetam administration. They found that levetiracetam failed to influence the concentrations of GABA, glutamate and glutamine in mouse brain following both single and repeated levetiracetam administration. Similarly levetiracetam was without effect on the activities of GABA-T and GAD. These results suggest that the GABAergic system is probably not involved in the mechanism of action of levetiracetam. In this thesis, the effect of levetiracetam on ECF concentrations of several excitatory and inhibitory amino acid neurotransmitters (see introduction) in the rat frontal cortex and the hippocampus was investigated by use of in vivo microdialysis model.

6.2 EXPERIMENTAL PROTOCOL

Amino acid concentrations in ECF were measured at the Brain Repair Centre, Cambridge. Male Sprague-Dawley rats (Charles River, Margate, Kent) weighing 300-350 g were used. Microdialysis probes were implanted in the frontal cortex and the hippocampus as previously described in Chapter 2. Two days after surgery, baseline samples were taken and the rats were administered I.P. with 80 mg/kg levetiracetam. Dialysate samples (30 μl) were collected at 15 minutes intervals for 2 hours and 30 minute intervals for a further 6 hours. Samples were stored frozen (-70°C) until required for the measurement of amino acid content.

6.3 RESULTS

6.3.1 ECF amino acids concentrations

Of the 19 amino acids measured in frontal cortex and hippocampal ECF, only two amino acids, namely taurine and glutamate, concentrations changed after levetiracetam (80 mg/kg) administration (Table 6.1). Taurine and glutamate concentrations significantly change in two brain areas (the results are expressed as percentage changes with respect to baseline). In this experiment, three baseline samples were collected in each experiment and mean sample values are shown. Mean ± s.e.m basal dialysate values for taurine in the frontal cortex and hippocampus were 5.91 ± 0.69 μM and 10.61 ± 0.59 μM respectively.

Table 6.1: Changes in ECF taurine concentrations, expressed as a % of baseline, after 80 mg/kg levetiracetam administration

Time (h)	Taurine		control	
	FC	HC	FC	HC
Baseline	100 ±6	100 ±7	100 ±7	100 ±13
0.3	59*±11	68*±15	115 ±12	110 ±9
0.7	68*±13	70*±11	140 ±16	119 ±12
1.0	58*±8	84*±10	113 ±13	110 ±15
1.3	67*±15	66 ±8	102 ±10	117 ±21
1.7	53*±12	63*±11	119 ±10	107 ±8
2.0	57*±13	62*±14	117 ±8	93 ±7
2.3	47*±8	70*±11	125 ±16	92 ±3
2.7	44*±9	62*±10	110 ±15	83 ±2
3.0	44*±11	72*±14	108 ±11	87 ±2
3.3	49*±8	64*±12	115 ±28	102 ±6
3.7	55*±8	79*±8	112 ±14	98 ±6
4.0	49*±6	62*±12	109 ±13	99 ±8
4.5	50*±9	62*±15	140 ±12	109 ±6
5.0	41*±5	54*±12	116 ±15	102 ±10
5.5	50*±12	57*±12	115 ±16	102 ±12
6.0	44*±7	60*±12	122 ±16	105 ±12
6.5	56*±9	55*±11	121 ±13	98 ±10
7.0	47*±11	60*±11	121 ±16	95 ±8
7.5	47*±5	50*±5	113 ±15	92 ±4
8.0	36*±5	56*±13	96 ±12	99 ±4

These data are mean data from 6 animals.
*$P < 0.05$ compared to saline controls (one way ANOVA)

Table 6.2: Changes in ECF glutamate concentrations, expressed as a % of baseline, after 80 mg/kg levetiracetam administration

Time (h)	Glutamate		control	
	FC	HC	FC	HC
Baseline	100 ±10	100 ±19	100 ± 2	100 ± 3
0.3	63 ± 5	60 ±14	97 ±17	73 ±16
0.7	79 ±14	64 ±16	118 ±13	70 ±14
1.0	80 ± 9	65 ±11	130 ±16	62 ±14
1.3	89 ±16	63 ±11	105 ±18	63 ±11
1.7	73 ± 8	58 ± 6	125 ±17	63 ±11
2.0	78 ±10	42 ± 3	97 ±16	65 ± 6
2.3	78 ± 9	64 ±11	101 ±16	57 ± 9
2.7	64 ± 9	58 ±12	99 ±21	64 ± 9
3.0	88 ±18	75 ±12	115 ±16	60 ±11
3.3	82*±13	54 ±12	118 ±20	61 ±11
3.7	65*±13	52 ± 9	130 ±17	58 ±6
4.0	59*±14	50 ±11	115 ±23	62 ±10
T **4.5**	63 ±20	47 ±11	126 ±23	56 ±12
5.0	69 ±18	53 ± 9	126 ±25	60 ±13
5.5	52*±11	39 ±14	118 ±21	66 ±18
6.0	49 ±18	52 ±10	113 ±17	79 ±19
6.5	53*± 3	66 ±20	124 ±21	69 ±12
7.0	43*±11	43 ±12	102 ±15	84 ±15
7.5	40*±15	52 ±11	124 ±17	73 ±12
8.0	45*±13	49 ±20	99 ±14	55 ± 7

These data are mean data from 6 animals.
*$P < 0.05$ compared to saline controls (one way ANOVA)

Figure 6.1 and 6.2 shows respectively ECF taurine concentrations over time in the frontal cortex and hippocampus after administration of 80 mg/kg levetiracetam. Compared to the saline control group, it can be seen that taurine concentrations are significantly reduced in both the frontal cortex and the hippocampus after levetiracetam administration. In the frontal cortex, a decrease was observed in ECF taurine concentrations which began gradual decline almost immediately after levetiracetam administration and finally decrease to 36 % of baseline at 8 hours. A similar pattern was seen in the hippocampus, with hippocampal ECF taurine concentrations decreased to 56 % of baseline at 8 hours.

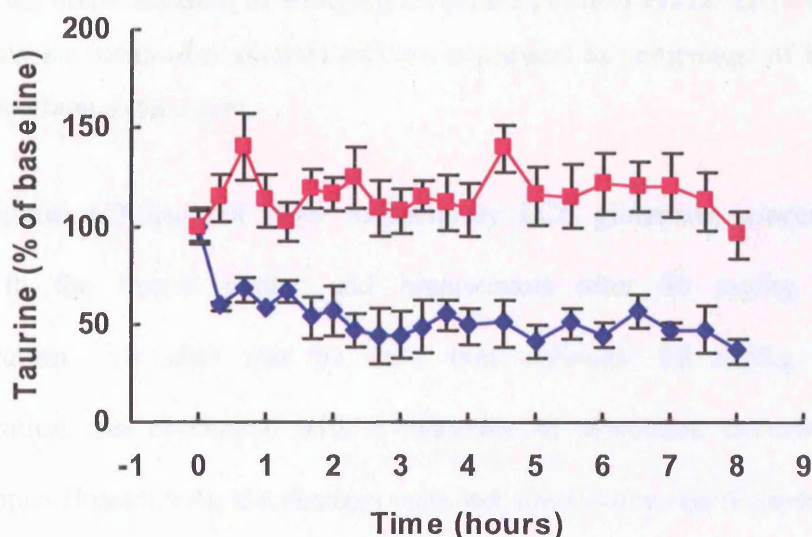


Figure 6.1: ECF taurine concentrations in the frontal cortex (◆) before after the administration of 80mg/kg levetiracetam or 0.9% saline (■ control). Values are mean of 6 animals and are expressed as percentage of baseline; vertical bars show s.e.m.

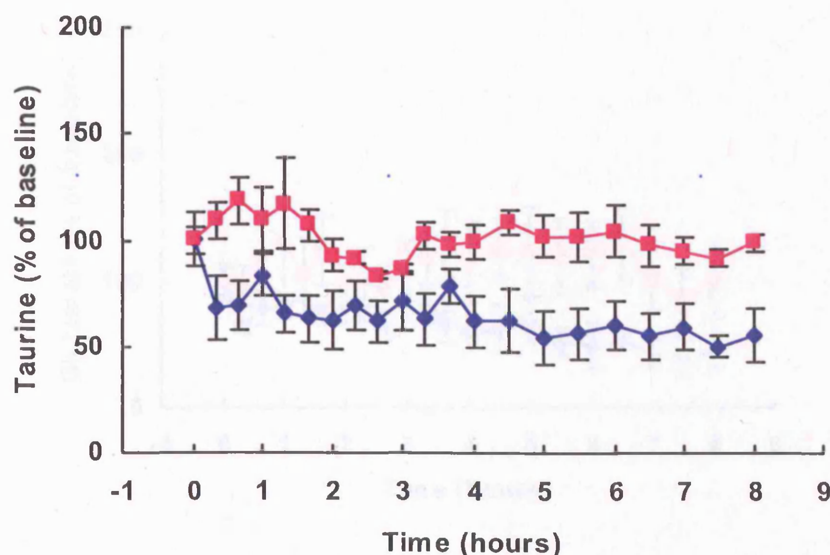


Figure 6.2: ECF taurine concentrations in the hippocampus (◆) before and after the administration of 80mg/kg levetiracetam or 0.9% saline (■ control). Values are mean of 6 animals and are expressed as percentage of baseline; vertical bars show s.e.m.

Figures 6.3 and 6.4 show respectively ECF glutamate concentrations time profiles in the frontal cortex and hippocmaus after 80 mg/kg levetiracetam administration. It also can be seen that although 80 mg/kg levetiracetam administration was associated with a reduction of glutamate concentration in the hippocampus (Figure 6.4), the changes were not statistically significance. In contrast, the frontal cortex ECF glutamate concentrations begin to decline after 3.5 hours and continued to decline to 45% of baseline at 8 hours after levetiracetam administration. These decreases were statistically significant (see Table 6.2).

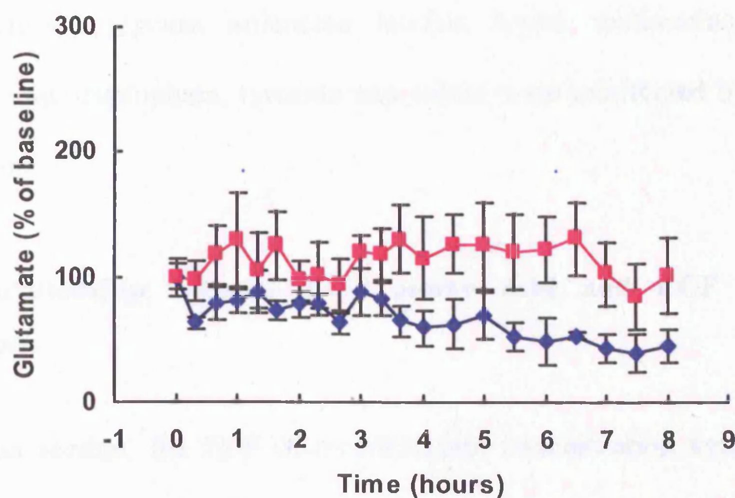


Figure 6.3: ECF glutamate concentrations in the frontal cortex (◆) before and after the administration of 80mg/kg levetiracetam or 0.9% saline (■ control). Values are mean of 6 animals and are expressed as percentage of baseline; vertical bars show s.e.m.

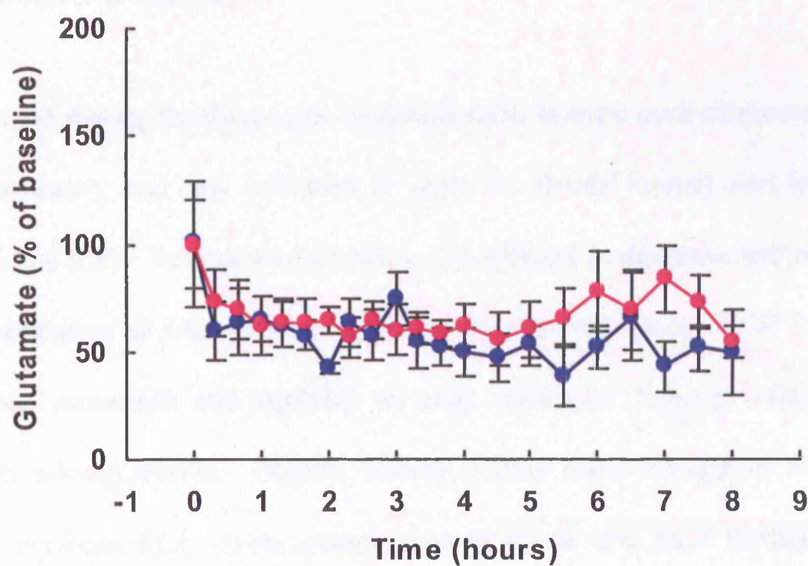


Figure 6.4: ECF glutamate concentrations in the hippocampus (◆) before and after the administration of 80mg/kg levetiracetam or 0.9% saline (■ control). Values are mean of 6 animals and are expressed as percentage of baseline; vertical bars show s.e.m.

ECF concentration for alanine, arginine, asparagines, aspartic acid, citrulline, GABA, glutamine, glycine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptopham, tyrosine and valine were unaffected by levetiracentam administration.

6.3.2 Relationship between ECF amino acid and ECF levetiracetam concentrations

In this section, the ECF of levetiracetam concentration versus time profiles presented in Chapter 4 after 80mg/kg levetiracetam administration are plotted with that of the ECF taurine and glutamate concentration versus time profiles that have been described in the previous section. The aim of this approach is to ascertain whether or not there is a relationship between levetiracetam concentration and amino acid concentrations in brain ECF.

After 80 mg/kg levetiracetam administration taurine concentrations decreased almost immediately and this occurred in both the frontal cortex and hippocampus (Figures 6.5 and 6.6). Taurine concentrations continued to decrease and reached their lowest concentration at 8 hours post levetiracetam administration. ECF Levetiraceam concentrations increased and reached to peak values at 3 hours after 80 mg/kg levetiracetam administration. Clearly, although there does not appear to be a direct relationship between ECF levetiracetam concentrations and ECF frontal cortex and hippocampal taurine concentrations, the observed changes can be attributed to levetiracetam. A similar pattern was also observed in relation to ECF frontal cortex glutamate concentration changes after levetiracetam administration (Figure 6.7).

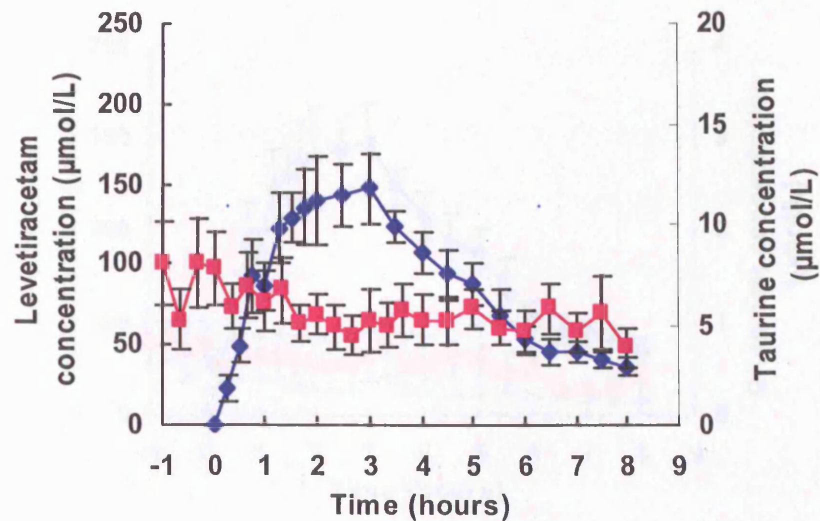


Figure 6.5: ECF concentration versus time profiles for levetiracetam (◆) and taurine (■) in the frontal cortex after 80 mg/kg levetiracetam administration. Values are mean ± s.e.m of 6 animals.

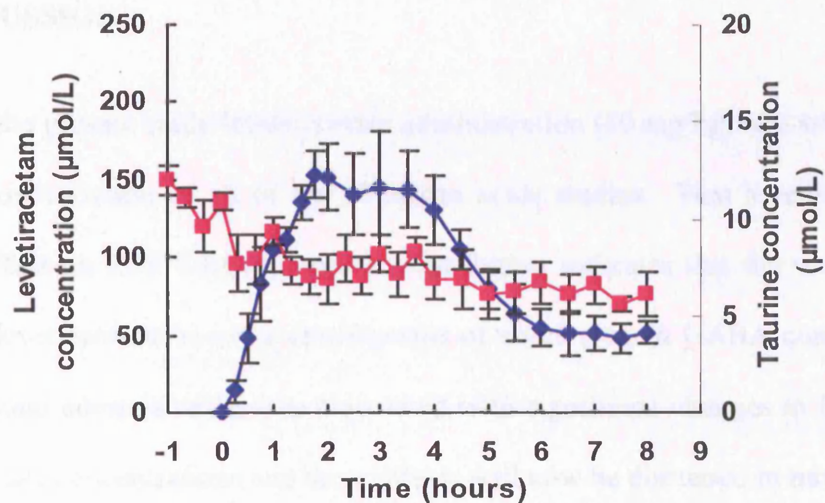


Figure 6.6: ECF concentration versus time profiles for levetiracetam (◆) and taurine (■) in the hippocampus after 80 mg/kg levetiracetam administration. Values are mean ± s.e.m of 6 animals.

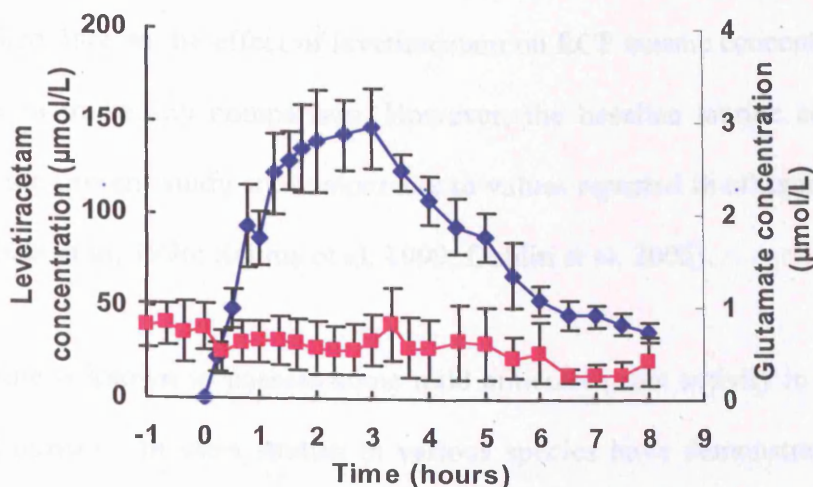


Figure 6.7: ECF concentration versus time profiles for levetiracetam (◆) and glutamate (■) in the frontal cortex after 80 mg/kg levetiracetam administration. Values are mean ± s.e.m of 6 animals.

6.4 DISCUSSION

In the present study levetiracetam administration (80 mg/kg) was without effect on ECF concentration of 18 of the 20 amino acids studies. That levetiracetam was without effect on ECF GABA concentration further indicates that the mechanism of action of levetiracetam is not a consequence of altering brain GABA concentrations. Levetiracetam administration was associated with significant changes in ECF taurine and glutamate concentrations and these effects will now be discussed in turn.

6.4.1 Taurine

Levetiracetam administration (80 mg/kg) was associated with a significant reduction in ECF taurine concentrations in both the frontal cortex and the hippocampus in present study. Taurine concentrations deceased at 1 hour after

levetiracetam administration and continued to decrease up to 8 hours. As there are no other published data on the effect of levetiracetam on ECF taurine concentrations, it is not possible to make any comparison. However, the baseline taurine concentration observed in the present study are comparable to values reported in other microdialysis studies (Wilson et al, 1996; Sejima et al, 1999; Dahlin et al, 2005).

Taurine is known to possess some mild anticonvulsant activity in both animal models and human. In vitro studies in various species have demonstrated that low levels of taurine are associated with epilepsy and other seizure disorders (Birdsall, 1998). Using rat cortex and hippocampus slices, an antiepileptic effect of taurine were investigated. The results showed seizure-like events were suppressed by taurine administration. Their findings suggest that taurine, as a glycine agonist, could serve as potential antiepileptic agents (Kirchner et al, 2003). A potential role of taurine on epilepsy were also studied in a rat seizure model. Taurine had a significant antiepileptic effect at 20, 40 and 80 mg/kg doses and the results indicated that taurine may play an inhibitory role against epilepsy as an inhibitory amino acid in CNS (Li et al, 2005). In a patient series, Wilson et al (1996) reported on twelve patients with epilepsy. ECF concentrations of eight amino acids were monitored during spontaneous seizures. They observed that ECF taurine concentrations were increased in the hippocampus of epileptic patients. Taurine-like compounds, such as taltrimide and MY103, have been used in clinical trials of epileptic patients. Other taurine structural analogues, such as phosphonates and guanidine derivatives, were also studied (Gupta et al, 2005).

The evidence above indicates that taurine and its analogues seem to possess antiepileptic activity. It is thus difficult to reconcile the decrease in brain taurine with the antiepileptic effect of levetiracetam. What is the mechanism by which levetiracetam reduces taurine levels? Changes in extracellular taurine concentrations occur with changes in osmolarity and neuronal swelling or shrinkage. In addition, neuronal depolarisation has been shown to increase taurine levels (Garcia Dopico et al, 2004). It is possible that the reduced taurine levels are not relevant to the antiepileptic action of levetiracetam, but are due to the antiepileptic action of levetiracetam (e.g. levetriacetam reduces neuronal depolarisation and so reduces extracellular taurine).

6.4.2 Glutamate

In the present study, although ECF glutamate concentrations decreased in both the frontal cortex and the hippocampus after 80 mg/kg levetiracetam administration, the changes were only statistically significant in the frontal cortex. Sills et al (1997) have reported on glutamate concentrations in the mouse brain after 300 mg/kg levetiracetam administration. Neither acute nor chronic (5 days) levetiracetam administration was associated with any changes in brain glutamate concentrations. The differences between the present study and that of Sills et al (1997) may be attributable to the fact that whole brain concentrations may not accurately reflect ECF concentrations. There is no patient or human volunteer studies published reporting the effect of levetiracetam in ECF, CSF or brain glutamate concentrations.

The mechanism by which ECF glutamate concentrations are changed by levetiracetam is unknown. Glutamate concentrations in ECF are low (3-4 μM) compared to concentrations in the intracellular fluid (ICF). Glutamate transporters

carry glutamate from the ECF to the ICF reuptake of glutamate into glial cells where glutamate is converted to glutamine which in turn is converted back into glutamate (Danbolt, 2001). Therefore, there are two possible mechanisms by which levetiracetam could decrease ECF glutamate concentrations. 1. Levetiracetam could decrease glutamate release. 2. Levetiracetam could increase glutamate uptake.

6.4.3 GABA and other amino acids

Levetiracetam did not affect ECF GABA or other amino acid concentrations in the present study. These results are in agreement with previous observations by Sills et al (1997), Poulain & Margineanu (2002) and Gower et al (1994). A lack of effect on mouse brain GABA concentrations and on the enzymatic activities of GABA-T and glutamic acid decarboxylase by levetiracetam was reported by (Sills et al, 1997). Extensive experiments on the receptor binding assay profile of levetiracetam indicated that levetiracetam was inactive at all of the more than 30 neurotransmitter receptors and ion channel sites, which indicated that the levetiracetam binding site in the brain was not directly related to any site that standard AEDs act upon (Noyer et al, 1995, Poulain & Margineanu, 2002).

6.4.4 Conclusions

1. Levetiracetam administration (80 mg/kg) was associate with significant reductions in ECF taurine concentrations in both the frontal cortex and the hippocampus. The mechanisms by which levetiracetam affects taurine or the role of taurine in the mechanism of action of levetiracetam is unknown.

2. ECF glutamate concentrations were also reduced by levetiracetam administration but in contrast to taurine, the reduction was only significant in the cerebral cortex. This effect may be via an increase in glutamate uptake or by decrease of glutamate release. Both these mechanisms could contribute to the antiepileptic action of levetiracetam.

3. ECF GABA and other amino acid concentrations were unaffected by levetiracetam administration providing further evidence that the anticonvulsant action of levetiracetam is unlikely to be mediated via an action on the GABAergic systems.

Chapter 7

The effect of vigabatrin and levetiracetam on *in vivo* paired-pulse inhibition

7.1 INTRODUCTION

Vigabatrin is effective in numerous animal seizure models including those induced by strychnine, isoniazid, low dose bicuculline, picrotoxin and pentylenetetrazol (Lewis & Wallace, 2001; Haugvicova et al, 2002). It is an efficacious AED in patients with intractable epilepsy (Turanli et al, 2006), but it may precipitate status epilepticus (de Krom et al, 1995). The explanation for the dose dependant effects of vigabatrin and why vigabatrin loses anticonvulsant effect at higher doses is unknown. It is possible that the increased extracellular GABA after vigabatrin administration, possibly reduces GABA release through activation of $GABA_B$ receptors. In addition GABA accumulation may desensitise $GABA_A$ receptors (Mott & Lewis, 1994). Using hippocampal slice preparations, vigabatrin has been shown to inhibit early $GABA_A$ receptor-dependent paired pulse inhibition in dentate and this disinhibition is thought to be due to activation of pre-synaptic $GABA_B$ receptors (Sayin et al, 1997). Vigabatrin also showed an effect reducing paired pulse inhibition in rat dentate gyrus *in vivo* and *in vitro*, which suggests that activation of presynaptic $GABA_B$ receptors by increased extracellular GABA may be one of the contributing factors to the apparent paradoxical effect of vigabatrin on synaptic inhibition (Sayin et al, 2001).

Although the binding site of levetiracetam in the CNS has been identified as synaptic vesicular protein 2a (SV2a) (Lynch et al, 2004, Lambeng et al, 2006), the mechanism by which binding to this protein mediates the anticonvulsant effect and whether this is the only mechanism of action for levetiracetam remains unclear. Previous studies have shown that levetiracetam does not bind to any neurotransmitter

receptors (Noyer et al, 1995) and no definite GABAergic mechanism has been identified (Margineanu & Wulfert, 1995). Levetiracetam has a neuroprotective function in animal models of status epilepticus (Gibbs et al, 2006; Willmore, 2005), but this relates to its antiepileptic effect is still not completely clear.

In this section, using perforant path stimulation the effects of vigabatrin and levetiracetam on paired-pulse inhibition *in vivo* were further investigated so as to the characterise mechanisms and to compare the action these two AEDs on GABAergic inhibition.

7.2 EXPERIMENTAL PROTOCOL

The methods used are those described by Albertson & Joy (1987) and Joy et al (1992) and have been detail in Chapter 2. Male Sprague-Dawley rats had bipolar stimulating electrodes implanted under halothane anaesthesia in the perforant path, and recording electrodes placed in the dentate granule cell layer. The electrodes were positioned so as to obtain a maximal population spike response at the lowest stimulus strengths. The electrodes were held in place with dental cement, and the rats were allowed to recover. Three days later, constant current paired-pulse stimulation was delivered at 0.1 Hz. After obtaining a consistent evoked response and stable population spike recordings, the stimulus intensity that produced the half maximal population spike amplitude was determined and was used for single or paired pulse recordings. Data were gathered before drug administration periods and during drug exposure periods. Vigabatrin (1000mg/kg) and levetiracetam (80mg/kg) were then constituted in saline and administered after a pre-drug period of 30 minutes recording. Subsequent recordings occurred for 5 minutes at the beginning of each 30 minute

period for a total of 8 hours. Finally a single point recording at 24 hour for 5 minutes was undertaken. A granule-cell excitability curve was obtained using pairs of stimuli of fixed intensity with interpulse intervals ranging between 20-100 ms. The paired pulse index was defined as the population spike amplitude resulting from the second pulse (p2) divided by the population spike amplitude resulting from the first pulse (p1). Since early paired pulse inhibition is dependent on size of conditioning pulse, the intensity of the stimulating pulse was adjusted, so that size of p1 remained constant throughout the experiment at half maximum. Paired-pulse ratio values were obtained using 8-10 pairs of stimuli at a fixed time interval. Paired pulse intervals of 20, 50 and 100 ms were used.

In previous paired pulse studies of perforant path, a chloride-dependent phase of inhibition occurred at 10-40 ms inter-pulse interval. These intervals reflect the recurrent or feed-forward stimulation of interneurones and resultant activation of fast chloride-mediated $GABA_A$ receptor inhibition. A facilitation at 40-100 ms intervals is observed (Oliver & Miller, 1985). Comparisons at these three intervals can be used to determine whether alterations in granule-cell excitability are associated with changes in inhibition or other processes (Joy et al, 1992).

7.3 RESULTS

7.3.1 Conditioning stimulus

In these experiments great care was taken to maintain a constant conditioning population spike amplitude. In animals, over the first 8 hours following vigabatrin and levetiracetam administration, the stimulus intensity had to be increased in order to

maintain the constant population spike. By 24 hours, however, the stimulus intensity to evoke an identical population spike to baseline was unchanged.

7.3.2 Paired-pulse inhibition and vigabatrin

It can be seen from Figure 7.1 that vigabatrin resulted in a decrease in paired pulse inhibition at the 20ms interpulse interval at 24 hours post vigabatrin administration.

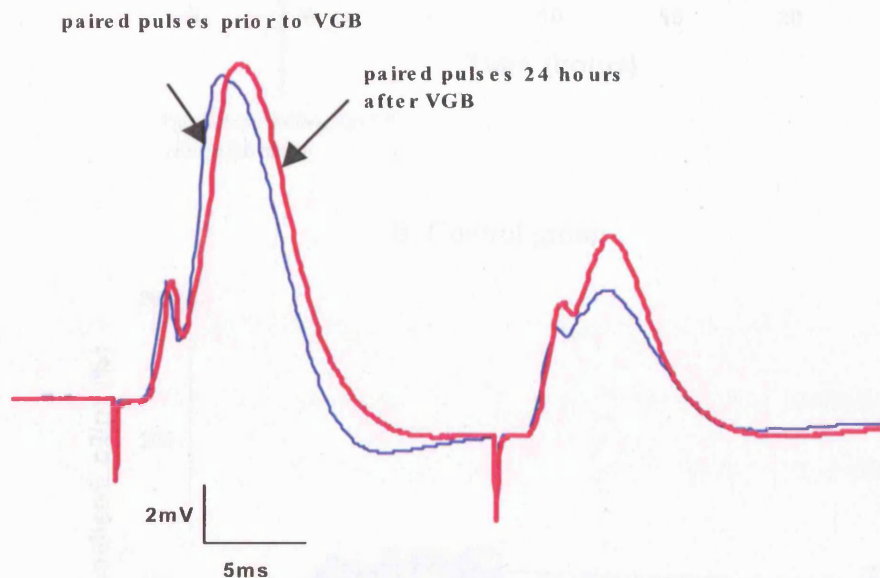
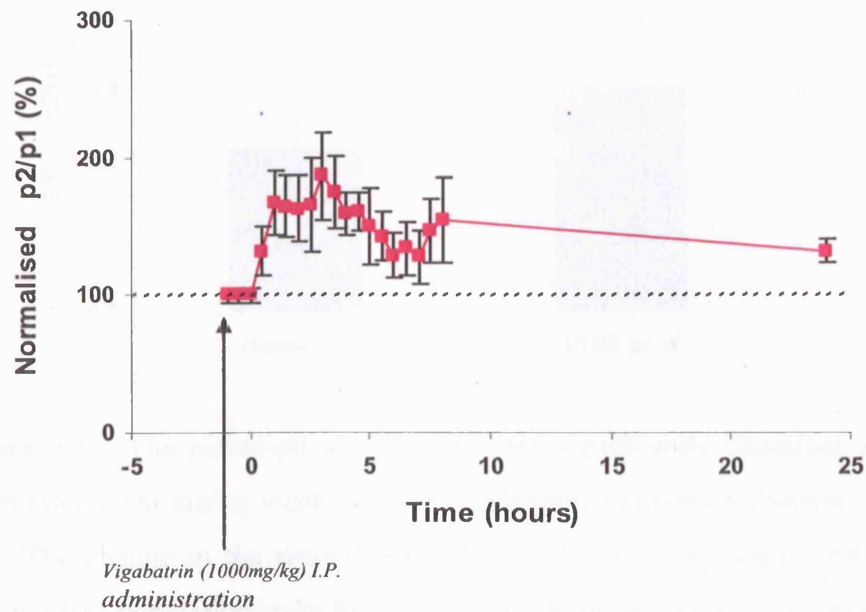


Figure 7.1: Paired-pulse inhibition was decreased at 20ms interval after 24 hours 1000mg/kg vigabatrin administration (–). The blue line (–) shows paired-pulse inhibition (PPI) before vigabatrin administration. Mean ± s.e.m for 6 animals. VGB= vigabatrin.

The maximum disinhibition occurred at approximately 1-4 hours and at 24 hours paired pulse inhibition was still significantly decreased ($P < 0.01$ 2 paired t-test) for the 20ms interpulse interval. In the control group, there was no change of paired pulse inhibition at any time point (Figure 7.2 A & B; Figure 7.3).

A: Vigabatrin group



B: Control group

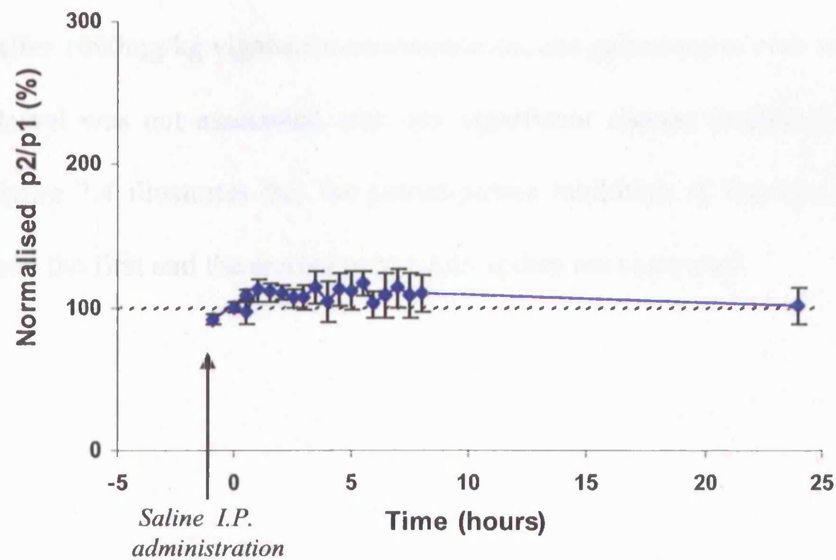


Figure 7.2: Paired-pulse inhibition (PPI) time profile after 1000mg/kg vigabatrin (A) and saline (B) administration. The percent change of PPI in the second population spike (p2) amplitude compared with the first population spike (p1) for 6 rats (mean ± sem) is presented at the 20 ms intervals. ….. indicates baseline values.

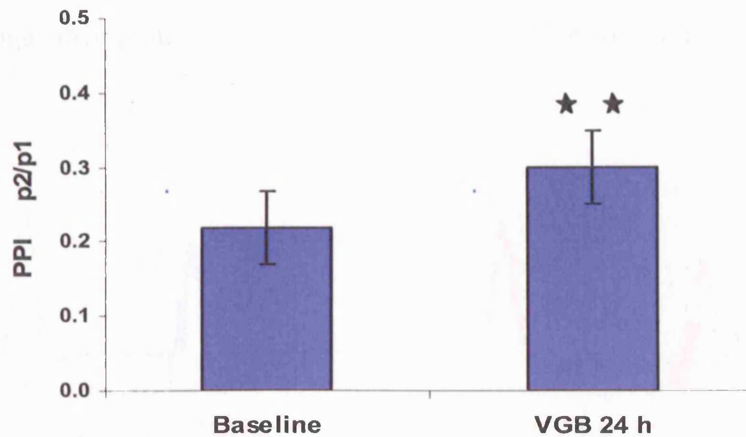


Figure 7.3: The paired-pulse inhibition was significantly decreased at 24 hours after 1000 mg/kg vigabatrin administration compared to baseline, $P < 0.01$. The change in the second population spike (p2) amplitude compared with the first population spike (p1) for 6 animals (mean ± sem) is presented at the 20 ms interval.

After 1000mg/kg vigabatrin administration, the paired-pulse ratio at 50ms and 100ms interval was not associated with any significant change compared to control group. Figure 7.4 illustrates that the paired-pulses inhibition in the two groups are similar when the first and the second population spikes are compared.

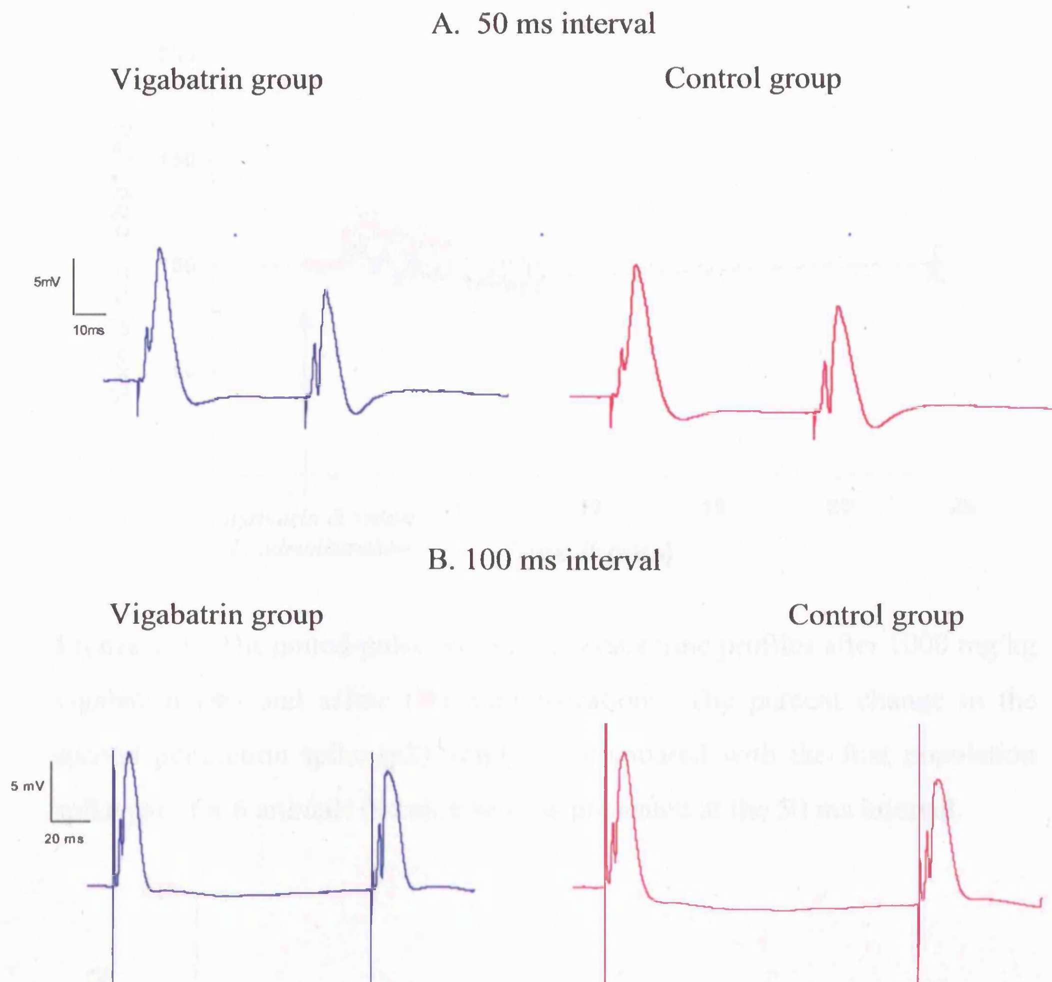


Figure 7.4: Effects of vigabatrin on paired-pulse inhibition (PPI) after 1000 mg/kg vigabatrin administration. The PPI was unchanged in the subsequent test population spikes (pink lines) compared with the baseline (blue lines). The interstimulus interval (ISI) in these traces was 50ms (A) and 100 ms (B). Bar = 5mV.

Figures 7.5 and 7.6 show respectively the percentage change in the second population spike (p2) amplitude compared with the first population spike (p1) at 50 ms and 100 ms. It is clear that there were no significant difference ($P > 0.05$) in paired-pulse inhibition between the vigabatrin (1000 mg/kg) administered groups and the control groups.

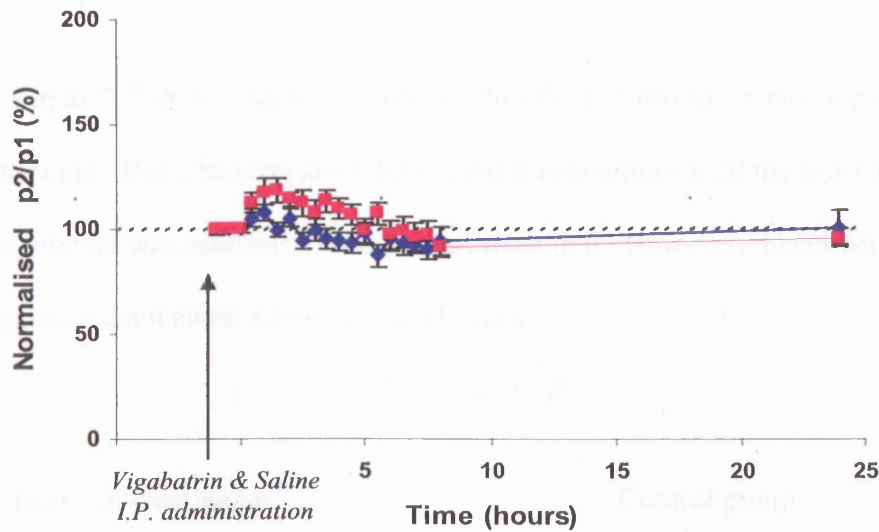


Figure 7.5: The paired-pulse inhibition versus time profiles after 1000 mg/kg vigabatrin (◆) and saline (■) administrations. The percent change in the second population spike (p2) amplitude compared with the first population spike (p1) for 6 animals (mean ± sem) is presented at the 50 ms interval.

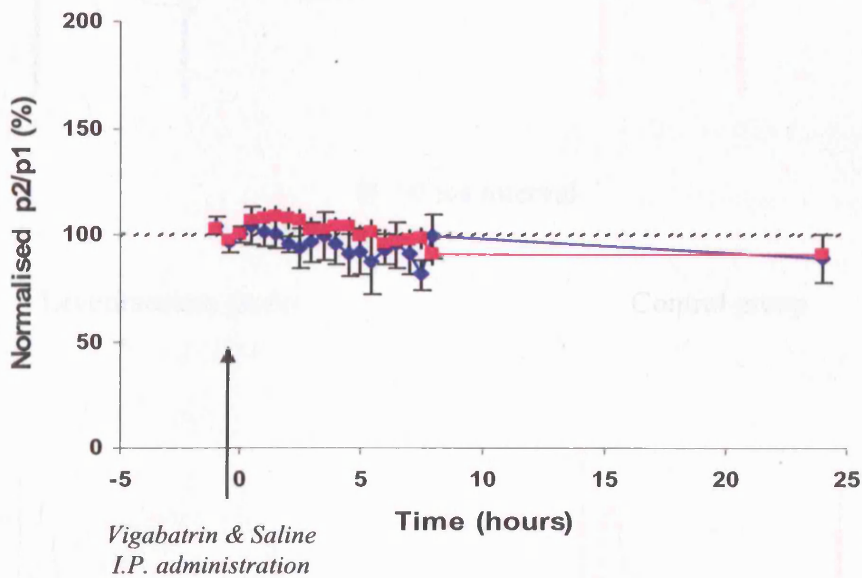


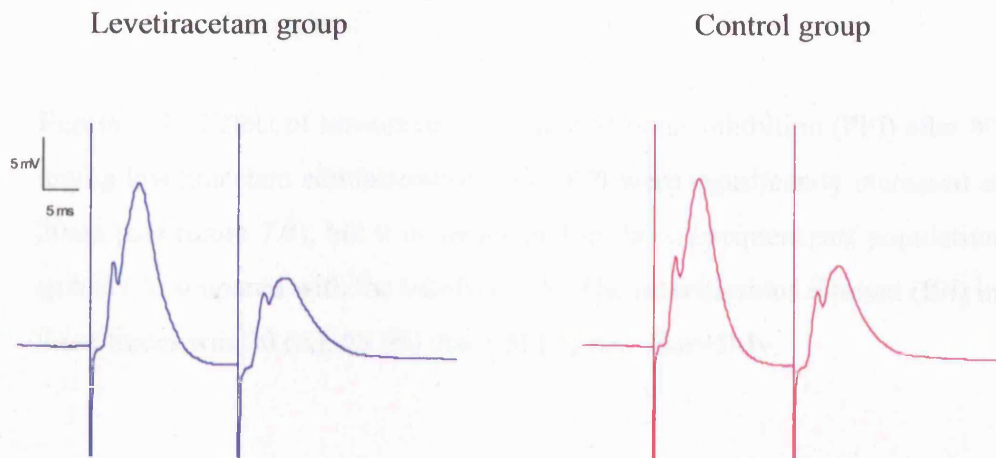
Figure 7.6: The paired-pulse inhibition versus time profiles after 1000 mg/kg vigabatrin (◆) and saline (■) administrations. The percent change in the second population spike (p2) amplitude compared with the first population spike (p1) for 6 animals (mean ± sem) is presented at the 100 ms interval.

7.3.3 Paired-pulse inhibition and levetiracetam

Figure 7.7 shows that time course of the effect of levetiracetam on paired-pulse inhibition. It can be seen that whilst there was no effect at 50 ms and 100 ms, the 20ms interval was associated with a small reduction. However, this effect in the dentate gyrus did not attain statistical significance.

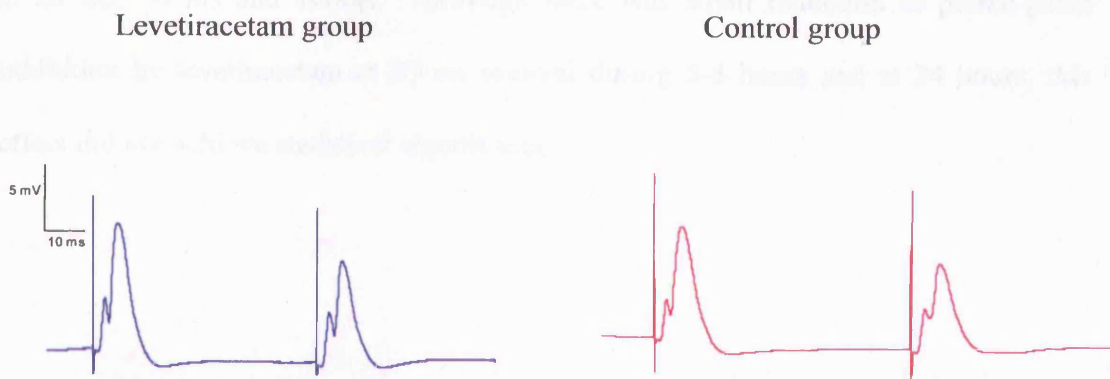
A. 20 ms interval

Levetiracetam group Control group



B. 50 ms interval

Levetiracetam group Control group



C. 100 ms interval

Levetiracetam group Control group

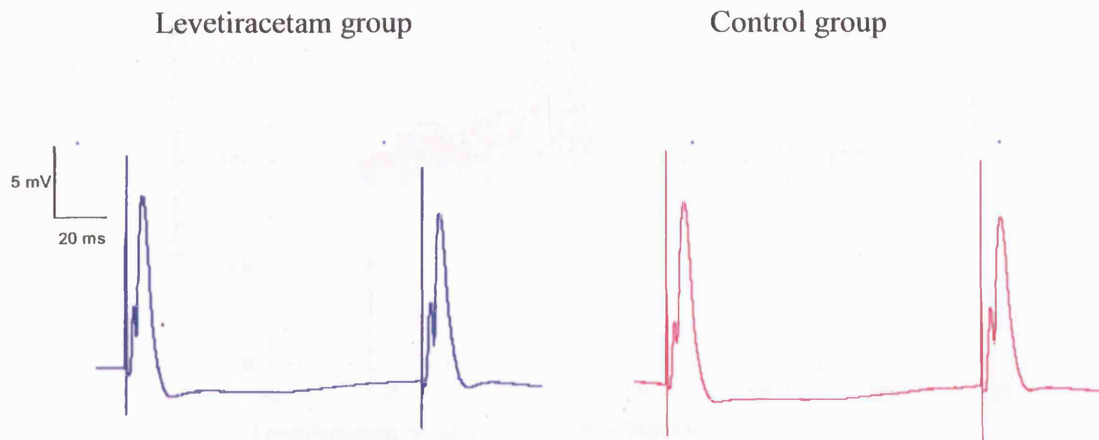


Figure 7.7: Effect of levetiracetam on paired-pulse inhibition (PPI) after 80 mg/kg levetiracetam administration. The PPI were significantly increased at 20ms (see figure 7.8), but it is unchanged in the subsequent test population spikes (-) compared with the baseline (-). The interstimulus interval (ISI) in these traces was 20 (A), 50 (B) and 100 (C) ms. Bar=5Mv.

Figures of 7.8, 7.9 and 7.10 show respectively the percentage change in the second population spike (p2) amplitude compared with the first population spike (p1) at 20 ms, 50 ms and 100ms. Although there was small reduction in paired-pulse inhibition by levetiracetam at 20 ms interval during 5-8 hours and at 24 hours, this effect did not achieve statistical significance.

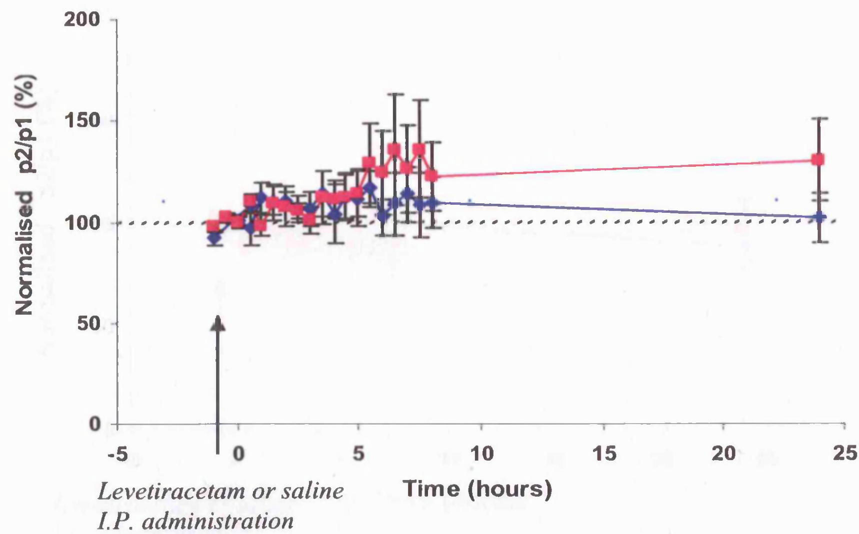


Figure 7.8: The paired-pulse inhibition versus time profiles after 80 mg/kg levetiracetam (■) and saline (◆) administrations. The percent change in the second population spike (p2) mean ± sem is presented at the 20 ms interval amplitude compared with the first population spike (p2) for 6 animals.

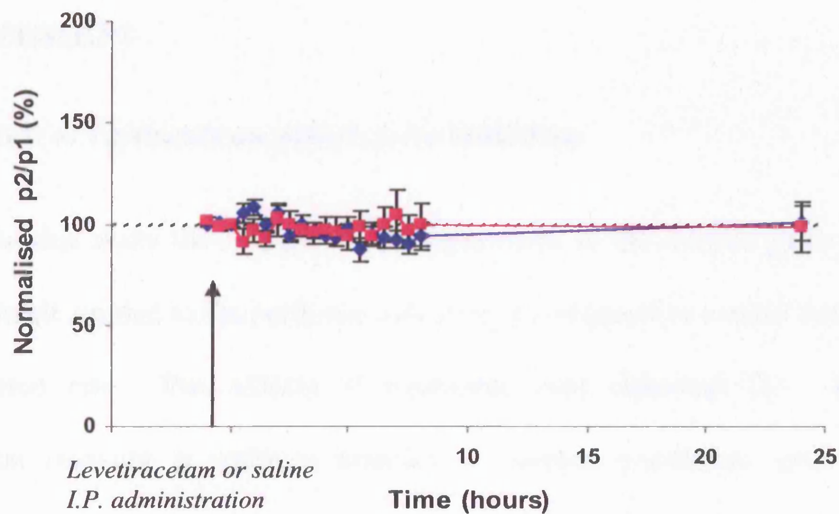


Figure 7.9: The paired-pulse inhibition versus time profiles after 80 mg/kg levetiracetam (■) and saline (◆) administrations. The percent change in the second population spike (p2) mean ± sem is presented at the 50 ms interval amplitude compared with the first population spike (p2) for 6 animals.

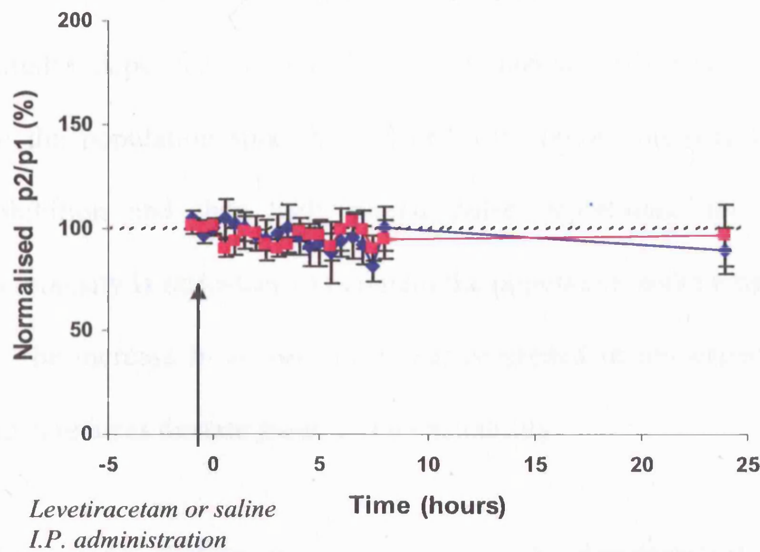


Figure 7.10: The paired-pulse inhibition versus time profiles after 80 mg/kg levetiracetam (■) and saline (◆) administrations. The percent change in the second population spike (p2) mean ± sem is presented at the 100 ms interval amplitude compared with the first population spike (p2) for 6 animals.

7.4 DISCUSSION

7.4.1 Effect of vigabatrin on paired-pulse inhibition

In this study the changes in field potentials in the dentate gyrus evoked by paired stimuli applied to the perforant path were investigated in control and vigabatrin administered rats. Two effects of vigabatrin were observed: (1). Increase in stimulation intensity in order to maintain a constant population spike following vigabatrin administration. (2). Decrease in paired pulse inhibition at the 20 ms interval, which was still apparent by 24 hours.

The stimulation intensity was adjusted in the present study to maintain a constant population spike in all animals. Previous studies have indicated that the

stimulation intensity may be an indicator of the activation state of the brain and the peak amplitudes depended nonlinearly on the intensity (Komssi et al, 2004). A decrease in the population spike by reduced stimulation intensity can affect feed-forward inhibition and thus early paired pulse depression, and thus increased stimulation intensity is necessary to maintain the population spike amplitude (Sayin et al, 2001). The increase in stimulation intensity needed in my experiments suggests that vigabatrin reduces dentate granule cell excitability.

The second finding in the present study demonstrated that vigabatrin administration was associated with a significant decrease in paired-pulse early inhibition at 20 ms for at least 24 hours compared to the control group, which may reflect a reduced feed-forward $GABA_A$ receptor mediated inhibition in the hippocampus (Sayin et al, 2001). The mechanisms behind this remains poorly defined, but two possibilities could explain these results: 1) Vigabatrin increased extracellular GABA concentrations and decreased GABA uptake resulting in activation of presynaptic $GABA_B$ receptors which decreased GABA release; 2) Extracellular GABA increases can also desensitise the post-synaptic $GABA_A$ receptor (Figure 7.11).

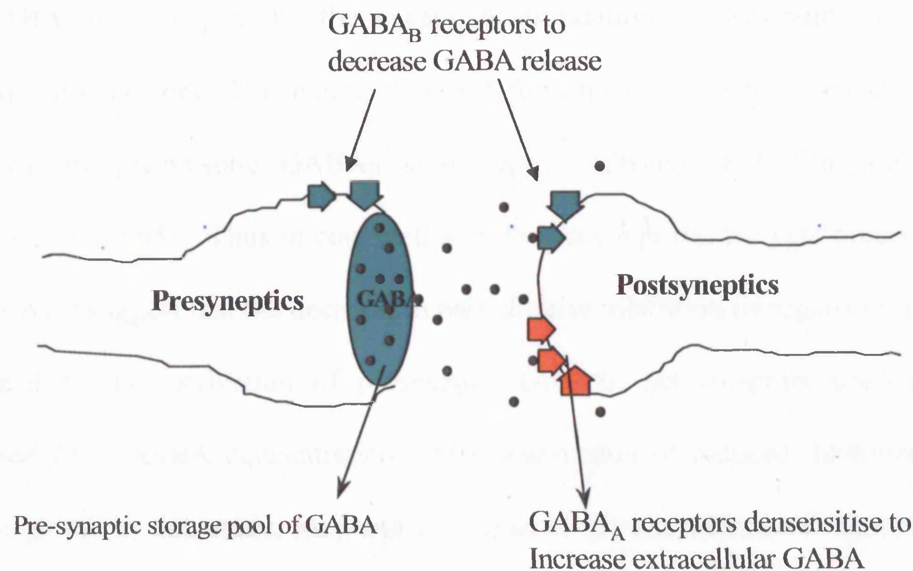


Figure 7.11: A schematic showing GABA$_A$ and GABA$_B$ receptors location on a pre- and postsynaptic neurone

The acute effects of vigabatrin decreasing paired-pulse inhibition at 20 ms interval could therefore be partially explained by a decrease in GABA uptake. Suzuki et al (1991) investigated that effect of vigabatrin on GABA uptake. They found that vigabatrin of 100-1000μM produced a concentration-dependent decrease in net uptake of ^{36}Cl in membrane vesicles, this suggested an effect of vigabatrin acting directly at the membrane vesicles rat GABA$_A$ receptor complex as a non-competitive antagonist. Vigabatrin was also reported to reduce activity-dependent suppression that occurs during a stimuli by reducing the function of presynaptic GABA$_B$ autoreceptors (Jackson et al, 2000). Using *in vivo* and *vitro* methods in the CA1 region of rat hippocampus, Sayin et al (2001) found that the decreased paired-pulse inhibition by vigabatirn was prevented or reversed by the co-application or subsequent application of GABA$_B$ antagonists saclofen or CGP 35348, which indicated that these paradoxical effects of vigabatrin may be attributed to the presynaptic inhibition of GABA uptake at

the GABAergic synapses by the excessive stimulation of presynaptically located $GABA_B$ autoreceptors. The release of GABA from interneurons has been shown to be decreased by presynaptic $GABA_B$ autoreceptors (Davies & Collingridge, 1993; Brucato et al, 1995). Thus in conjunction with these reports, the data present in this thesis would suggest that the decrease in paired-pulse inhibition by vigabatrin might be explained by the activation of presynaptic $GABA_B$ autoreceptors consequent to increased ECF GABA concentrations. The observation of reduced inhibition in the dentate gyrus, in this study, may explain some of adverse effects of vigabetrin, and could contribute to the paradoxical proconvulsant effects of vigabatrin in generalized seizures.

An alternative explanation is that increased GABA results in $GABA_A$ receptor desensitisation. Vigabatrin does not have a direct antagonistic effect on $GABA_A$ receptors, as shown by binding studies (Jackson et al, 1994a). However GABA-T inhibition produced by vigabatrin can be expected to increase inhibition postsynaptically by elevation of GABA concentrations in the brain. Overstreet & Westbrook (2001) examined the effects of vigabatrin on GABAergic synaptic transmission in hippocampal slices. Vigabatrin unexpectedly reduced miniature and evoked inhibitory postsynaptic currents and these effects were accompanied by an increase in tonic $GABA_A$ receptor-mediated current. The increase in tonic inhibition described in this study would explain the decrease in granule cell excitability that I observed. The gradual GABA accumulation in extracellular compartments led to a reduction in postsynaptic receptor sensitivity.

7.4.2 Effects of levetiracetam on paired-pulse inhibition

In this study, levetiracetam did not cause a statistically significant change in paired-pulse inhibition, but there was a slight tendency for a decrease in inhibition at 20ms. These data are in agreement with previous animal studies in different models (Margineanu & Wulfert, 1995; Margineanu & Klitgaard, 2003) that have shown that levetiracetam did not affect paired-pulse inhibition in the dentate gyrus at short intervals. Furthermore, levetiracetam did not alter the paired-pulse inhibition when bicuculline, a $GABA_A$ antagonist was administered. These data were considered to suggest that the anticonvulsant mechanisms of action of levetiracetam are not mediated by $GABA_A$ receptors. Recently Margineanu & Klitgaard (2003) also investigated the effect of levetiracetam on the paired-pulse interaction in the urethane-anesthetized animal model. Their results showed that levetiracetam had no significant effect on paired-pulse interaction in this model, similar to my results.

7.4.3 Conclusion

1. Vigabatrin has two effects on perforant path to dentate granule cell responses in this study: (1). Increase in stimulation intensity in order to maintain a constant population spike. (2). Decrease in paired-pulse inhibition of 20 ms interval, which was still apparent by 24 hours. These results can be explained on the basis of two mechanisms. Firstly, vigabatrin increased ECF GABA concentrations and decreased GABA uptake results in activation of presynaptic $GABA_B$ receptors which reduced GABA release; Secondly, the increased GABA concentration can desensitise post-synaptic $GABA_A$ receptors, but activate extrasynaptic $GABA_A$ receptors leading to reduced granule cell excitability. Vigabatrin had a predominant effect on the

inhibitory system resulting in disinhibition in the paired-pulse experimental paradigm, which would suggest that $GABA_A$ receptor-mediated feedback or forward inhibition does not underlie its action as an anticonvulsant, but that vigabatrin's antiepileptic effect may be mediated by $GABA_B$ receptor and extrasynaptic $GABA_A$ receptor activation.

2. Levetiracetam had no effect on excitability or paired-pulse inhibition occurring at 20-100 interstimulus intervals. These results are in agreement with previous studies, which suggest that the anticonvulsant activities of levetiracetam are probably mediated via a non-GABAergic mechanism.

Chapter 8

General discussion

8.1 INTRODUCTION

Approximately 80% of patients with epilepsy are rendered seizure-free with the long-established generally available AEDs (carbamazepine, ethosuximide, phenobarbital, primidone and valproate). However these patients may experience numerous drug-related problems such as idiosyncratic reactions and acute and chronic CNS adverse effects. For the remaining 20% of patients with severe-treatment-refractory epilepsy, little benefit is achieved from the use of these AEDs as intolerable adverse effects occur. Therefore there has been a need for new AEDs with improved risk-benefit ratios (i.e. greater efficacy and less toxicity) and simple pharmacokinetic characteristics and there has been a desire to understand better the mechanism of drug action so that more rational prescribing could be entertained. Indeed, since 1989, nine new AEDs (gabapentin, lamotrigine, levetiracetam, tiagabine, topiramate, oxcarbazepine, vigabatrin and felbamate and zonisamide) have been licensed for clinical use and despite significant effort their mechanisms of action are not fully understood. Similarly their pharmacokinetic/neuropharmacokinetic profiles are also not fully understood. Therefore the present thesis sought to investigate the mechanism of action and the pharmacokinetic/neuropharmacokinetic inter-relationship of two of the new AEDs, namely vigabatrin and levetiracetam.

8.2 PHARMACOKINETIC/NEUROPHARMACOKINETIC INTER-RELATIONSHIP

8.2.1 Vigabatrin

Although the blood pharmacokinetics of vigabatrin in man is well characterised, the central brain neuropharmacokinetics of vigabatrin are poorly

understood in that its CSF kinetics comprise of CSF analysis of single lumber puncture samples and there have been no studies of the ECF neuropharmacokinetics of vigabatrin. In relation to the rat, there are even sparser data. Perhaps the reason for the lack of data can be attributable to the notion that since vigabatrin continues to exert its pharmacological effect long after vigabatrin is eliminated from the blood/brain, an understanding of its kinetic profile would not significantly enhance the therapeutic use of vigabatrin. However, data presented in this thesis show that the pharmacokinetic/ neuropharmacokinetic inter-relationship of vigabatrin in the rat is not what one would anticipate. Thus whilst the serum pharmacokinetics of vigabatrin are linear and dose-dependent, and indeed its neuropharmacokinetics in the CSF and ECF frontal cortex and hippocampus are similarly linear and dose dependent, the kinetic inter-relationship between the three compartments is unexpected.

The rate of drug penetration into the CSF compartment is considered to be determined by essentially three physiochemical characteristics; ionisation, lipid solubility and plasma protein binding. In relation to AEDs, lipid solubility appears to be a major determinant. The blood protein binding of a drug determines the distribution of the drug in the body. Thus with highly protein bound drugs distribution is limited whilst for minimally bound drugs distribution is more extensive. Furthermore the non-protein bound component is available to transfer across the blood brain barrier and to enter the CSF and brain compartments and for many AEDs (e.g. phenytoin and lamotrigine) there is good correlation between free concentration in blood with that of CSF and brain tissue. With regards to vigabatrin, data presented in this thesis confirm that, as previously reported, it is not bound to proteins in serum and CSF vigabatrin concentrations did not reflect free drug concentrations in serum.

A possible explanation for this observation may be the fact that in the choroid plexus interface between the CSF and blood compartments there is an active organic acid transporter system, which is capable of driving CSF-borne organic acids into the blood stream and may also extrude vigabatrin (Misra et al., 2003). A further explanation may be that vigabatrin is not lipid-soluble, and requires active transport into the CSF compartment.

Because ECF vigabatrin concentrations in brain frontal cortex and hippocampus are adjusted concentrations based on in vitro microdialysis recovery values it is not appropriate to make direct comparisons with that of absolute concentration measurements in serum and CSF. Nevertheless, it is valid to compare vigabatrin concentrations in ECF of the frontal cortex and hippocampus. In this regard, vigabatrin distribution in ECF was brain region specific in that vigabatrin concentrations in the frontal cortex were 2-fold greater compared with concentrations achieved in the hippocampus. Such specific brain region distribution of AEDs has also been reported for phenytoin and lamotrigine (Walker et al., 1996; 2000). Interestingly, the fact that vigabatrin $t_{1/2}$ values are essentially identical for hippocampus (2.4-3.6 hours) and frontal cortex (2.5-3.6 hours) would suggest that the elimination processes in the two brain regions are similar. This suggests that there must be differences in the ability of vigabatrin to enter distinct brain areas.. To date, however, there have been no published data on the mechanism by which this difference may occur.

8.2.2 Levetiracetam

As with vigabatrin, the blood pharmacokinetics of levetiracetam in man is

well characterised but the central brain neuropharmacokinetics of levetiracetam are poorly understood. Although there are no reports on the brain distribution of levetiracetam, levetiracetam is reported to readily and rapidly enter the CSF compartment of patients with epilepsy with T_{max} values of 3-5 hours (Edwards et al., 2004). In relation to the rat, there is a single study whereby the kinetic inter-relationship between the blood and CSF compartments were compared (Doheny et al., 1999). Levetiracetam was observed not to bind to blood proteins and CSF concentrations reflected free levetiracetam concentrations in serum. However, the efflux of levetiracetam from the CSF compartment was significantly slower than that from the blood compartment (mean $t_{1/2}$ range: 4.4-4.9 hours and 1.8-2.8 hours respectively). Levetiracetam serum pharmacokinetic data presented in this thesis confirm that reported by Doheny et al (1999). In addition, the neuropharmacokinetic profiles of levetiracetam, as assessed by microdialysis monitoring of ECF, in the frontal cortex and hippocampus are observed to be essentially identical and that levetiracetam distribution in the brain is not brain region specific. This may be attributable to the fact that brain uptake of levetiracetam is not dependent on PgP or MRP transporters which may be expressed differentially in different areas of the brain (Potschka et al., 2004).

8.3 THE EFFECT OF VIGABATRIN ON CSF AMINO ACID NEUROTRANSMITTER CONCENTRATIONS

Because the CSF compartment is considered to reflect events in the brain, many studies have been undertaken whereby measurement of neurotransmitter candidates in CSF have been used as an index of neuropathology or the mechanism of action of a drug. Furthermore, since the CSF compartment is considered to be

kinetically indistinguishable from the site of action of drugs that have an effect in the CNS, correlation of CSF drug concentrations with that of CSF neurotransmitter concentrations have been used to explain putative mechanisms of drug action.

Vigabatrin has been described as a 'designer drug' because it was specifically developed so that it affects the GABA system and increases brain GABA concentrations; low GABA concentrations having been clearly associated with seizure occurrence. That vigabatrin increases CSF GABA concentrations has been confirmed in both human (Ben Menachem et al., 1988, 1991) and animal studies (Piredda et al., 1987; Bernasconi et al., 1988). In the present thesis, because of technical difficulties it was not possible to measure GABA in the CSF. Nevertheless, homocarnosine concentrations were measured and as homocarnosine is a major GABA conjugate, concentrations can be used as an indirect index of GABA concentrations. Indeed in this thesis vigabatrin administration was associated with significant increases in CSF homocarnosine concentrations. In addition, CSF taurine concentrations increased whilst a concurrent decrease in arginine and tyrosine concentrations was observed. The significance of these amino acid changes with regards to the mechanism of action of vigabatrin is not known.

8.4 THE EFFECT OF VIGABATRIN AND LEVETIRACETAM ON ECF BRAIN AMINO ACID NEUROTRANSMITTER CONCENTRATIONS

The ECF is the liquid compartment where the traffic of compounds and the exchange of chemical information between cells take place. Consequently, monitoring of the ECF by microdialysis is considered to reflect synaptic events at the site of action or biophase of drugs and in this thesis ECF amino acid concentrations

were measured in the frontal cortex and hippocampus after vigabatrin and levetiracetam administration.

8.4.1 Vigabatrin

Interestingly, after vigabatrin administration more amino acids were associated with a concentration change in ECF compared to that in CSF. This would suggest that discrete changes in specific areas of the brain may not be reflected in CSF whose composition is a composite of the whole brain. The most significant changes in ECF amino acid concentrations related to GABA, which increased by more than 6-fold, and glutamate, which decreased by 37-57%. Both of these changes are compatible with an anticonvulsant effect. What is particularly noteworthy is the observation that whilst ECF glutamate concentrations decreased in both the frontal cortex and the hippocampus, ECF GABA concentrations were only significantly elevated in the frontal cortex. Thus there appears to be regional specificity in relation to the pharmacological effect of vigabatrin on GABA.

8.4.2 Levetiracetam

In contrast to vigabatrin, levetiracetam was without effect on ECF GABA concentrations and these data, along with those of Sills et al (1997) who were unable to observe an effect of levetiracetam on catabolic and anabolic GABA enzymes, would suggest that indeed levetiracetam does not exert its anticonvulsant effect via an action on the GABA system. ECF taurine concentrations in both the frontal cortex and hippocampus were significantly reduced (16-64%).

8.5 RELATIONSHIP BETWEEN VIGABATRIN ECF NEUROPHARMACOKINETICS AND GABA CONCENTRATIONS

The regulation of GABA has been a particularly attractive target as a mechanism of action of new AEDs. Many new AEDs (e.g. vigabatrin, gabapentin, tiagabine, felbamate, oxcarbazepine and topiramate [Fish et al., 2003]) are considered to at least in part act in this manner. In the present thesis ECF GABA concentrations were used as a pharmacodynamic end point and their inter-relationship with that of the ECF AED concentrations were determined with a view to ascertaining whether or not there is a direct correlation between the two parameters.

After intraperitonial administration, vigabatrin rapidly entered the ECF compartment and peaked at approximately 1 hour after vigabatrin administration. ECF vigabatrin concentrations then declined exponentially. This neuropharmacokinetic profile was apparent in both the frontal cortex and the hippocampus. However, peak ECF vigabatrin concentrations in the frontal cortex were twice those seen in the hippocampus (113 μmol/L versus 52 μmol/L; Tables 3.3 and 3.4). Furthermore, when the ECF vigabatrin concentration versus time profile is compared to the ECF GABA concentration versus time profile, it can be seen that whilst ECF GABA concentrations in the hippocampus were unaffected (Figure 5.21), ECF GABA concentrations in the frontal were significantly elevated (Figure 5.20). However, the elevation in frontal cortex GABA concentrations did not parallel the ECF vigabatrin concentrations and indeed GABA concentrations only began to increase at approximately 3 hours post vigabatrin administration when in fact ECF vigabatrin concentration were in decline (concentrations were approximately 50% those achieved at C_{max}). Also, by 8 hours post vigabatrin administrations, when ECF

vigabatrin concentrations were at their lowest, ECF GABA concentrations were still increasing. These data would suggest that there is a lack of concordance between ECF vigabatrin concentrations and ECF GABA concentrations and extend data presented in other studies whereby a lack of concordance between CSF GABA and blood vigabatrin concentrations have been reported (Ben-Menachem et al., 1989, 1991). This delayed affect on GABA would be in line with the clinical observation that there is a time lag between ingestion of vigabatrin and achieving a clinical response.

A particular interesting observation, however, is that in the present thesis ECF GABA concentration were unaffected in the hippocampus even though vigabatrin distributed readily in the hippocampus; albeit ECF vigabatrin concentration were approximately 50% those achieved in the frontal cortex. Possible explanations for this include: (1) that a threshold vigabatrin concentration (> 50 μmol/L) needs to be achieved before significant GABA-T inhibition occurs, and (2) that hippocampal GABA-T has a different *Ki* to that that occurs in the frontal cortex.

In the present thesis it was not possible to show that ECF GABA concentrations increase dose-dependently since only one dose was investigated (1000 mg/kg). Previous studies, however, have shown that CSF GABA concentrations increase dose-dependently (Ben-Menachem et al., 1989; Riekkinen et al., 1989b; Erdal et al., 1999) and serial NMRS has shown very clearly that brain GABA concentrations increase dose-dependently over a dose range up to 3 g/day (Petroff et al., 1996c). Interestingly, it has been reported that clinically there is a ceiling above which increasing the dose of vigabatrin is without effect with regards to increasing the efficacy of the drug (McKee et al., 1993). An explanation for this clinical

observation relates to the fact that although brain GABA concentrations increase dose-dependently there is a ceiling (3 g/day) above which increasing the dose of vigabatrin is not associated with any further increase in GABA concentrations (Petroff et al., 1996c). That ECF GABA concentrations were not affected by vigabatrin in the hippocampus may suggest that there may be a lower dose (ECF concentration) which has to be achieved before GABA-T inhibition is significant and would result in increased GABA concentrations.

8.6 EFFECTS OF VIGABATRIN AND LEVETIRACETAM ECF ON PAIRED-PULSE INHIBTION

Data presented in this thesis clearly show that vigabatrin administration was associated with a reduction in paired-pulse inhibition recorded in the rat dentate gyrus evoked by perforant path stimulation at 20 ms interpulse interval but not at 50 ms and 100 ms interpulse intervals. As paired-pulse responses at 15-25 ms interpulse intervals are sensitive to $GABA_A$ receptor inhibition, it would suggest that vigabatrin may act by (a) a direct effect of vigabatrin on postsynaptic $GABA_A$ receptors via increased ECF GABA concentrations, (b) a presynaptic effect on $GABA_B$ receptors which serves to regulate GABA release, or (c) by other electrophysiological effects of vigabatrin as yet not defined. These data are in agreement with those reported by Sayin et al. (2001).

Vigabatrin increases ECF GABA concentrations by at least two mechanisms. The primary mechanism is through its irreversible binding to the enzyme GABA-T that is responsible for the breakdown of GABA in neurones and glia. The secondary mechanism relates to the ability of vigabatrin to block the uptake of GABA from the

synaptic cleft. The lack of an increase in ECF GABA levels noted in the microdialysis studies may not directly relate to GABA in the synaptic cleft, because: 1) increased GABA release or increased GABA present in the synaptic cleft may be removed by glial and extrasynaptic neuronal GABA transporters or 2) there may be only subregional or subcellular specific increases in GABA whilst microdialysis samples over a larger region. The possible increased GABA concentrations associated with vigabatrin administration are expected to have effects at both $GABA_A$ and $GABA_B$ receptors. An effect on presynaptic $GABA_B$ receptors might reduce the efficacy of inhibitory synaptic transmission and may explain the paradoxical proconvulsant effects of vigabatrin that have been reported in patients with generalized seizures.

Using anaesthetized rats, Margineanu and Wullfert (1995) were able to show that levetiracetam was without effect on paired-pulse inhibition. These data are confirmed in the present thesis using freely moving rats. These data suggest that levetiracetam has no direct action on $GABA_A$ receptors.

8.7 SUMMARY AND FURTHER WORK

This thesis describes various studies designed to ascertain the pharmacokinetic and neuropharmacokinetic inter-relationship of vigabatrin and levetiracetam in a freely moving and freely behaving rat model and their effects on CSF and brain ECF amino acid neurotransmitters. Studies exploring a more detailed understanding of the mechanism of action of vigabatrin and levetiracetam were also undertaken using paired-pulse inhibition recorded in the rat dentate gyrus and evoked by perforant path stimulation.

However, further studies are needed to extend the finings reported in this thesis: (1) In terms of the brain neuropharmacokinetics of vigabatrin and levetiracetam, other brain regions apart from the frontal cortex and hippocampus, should be investigated so as to establish the exact drug distribution in the brain ECF compartment; (2) To ascertain whether or not the differential effect of vigabatrin on ECF GABA concentrations extends to other brain regions; (3) To determine the GABA versus time profile beyond 8 hours post vigabatrin administration so as to ascertain the exact magnitude and time course of the GABA concentration changes that were observed in the frontal cortex; (4) To determine whether or not ECF GABA concentration changes are vigabatrin dose and concentration (ECF) dependent. (5) Further mechanism studies for vigabatrin and levetiracetam, especially given the novel binding site now identified for levetiracetam.

Other neurotransmitters and biologically active substances are undoubtedly also important in epileptogenesis. Nitric oxide (NO) plays a critical role in signal transduction in the CNS with major functions. It was recently shown that levetiracetam increase the expression of inducible NO in cultured astrocytes. The effect of levetiracetam on NO is due to an interaction with NMDA and AMPA pathways (Dagonnier et al, 2005). Levetiracetam increased the baseline production of NO after 20 minutes administration. The pre-treatment with levetiracetam induced an NMDA-mediated NO increase and these increases were dose related. In brain synapses, NO synthase activation is coupled to NMDA-mediated calcium entry at postsynaptic densities through regulatory protein complexes, and NMDA/NO inhibits the release of pre-synaptic glutamate (Sequeira et al, 2001). The inhibitory retrograde

action of postsynaptic NO on presynaptic terminals might also contribute to the mechanism of action of levetiracetam. Therefore, the further study on ECF NO concentrations in different brain region after levetiracetam or vigabatin administrations should also be considered.

Chapter 9

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