INVESTIGATION OF THE \textit{IN VIVO} PHARMACOLOGICAL PROPERTIES OF LAMOTRIGINE (LAMICTAL\textsuperscript{TM}) IN EPILEPTIC AND NON-EPILEPTIC RATS.

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Abstract

• ABSTRACT •

There is compelling evidence to suggest that epileptic seizures may be associated with neurochemical imbalances, leading to long-term neuronal excitability and the generation of epileptiform activity. Consequently, agents capable of stabilising hyperexcitable neuronal membranes may be effective for the management of epilepsy.

Lamotrigine (Lamictal™; LTG) is a novel anticonvulsant, considered to act as a use- and voltage-dependent inhibitor of type IIa Na⁺ channels, thereby stabilising excitable neuronal membranes, and reducing pathologically-altered neurotransmission.

In view of these findings, intracerebral microdialysis has been used to show LTG to specifically and significantly inhibit the Na⁺-dependent (veratridine-evoked), rather than basal- or Ca²⁺-dependent (elevated K⁺-evoked), efflux of amino acids, predominantly glutamate, from the ventral hippocampus of non-epileptic rats, both acutely and chronically (up to 21 days), at therapeutically relevant doses.

However, unlike LTG, the well-established Na⁺ channel modulators phenytoin and carbamazepine, agents with which LTG has been likened and shown to resemble in experimental seizure models, failed to significantly alter veratridine-evoked glutamate efflux, exhibiting a somewhat different neurochemical profile to that of LTG.

Additionally, a number of clinical studies have shown LTG to be effective at ameliorating non-convulsive absence and partial seizures, proving to be of particular benefit in combination with sodium valproate - possibly due to manipulation of opposite sides of the neurochemical balance, with sodium valproate enhancing GABAergic inhibition and LTG inhibiting excitation. Sub-chronic (5 days) LTG-administration failed to suppress electroencephalographic spike-and-wave discharges from the ventrolateral thalamus of Genetic Absence Epilepsy Rats from Strasbourg (an experimental correlate of the human condition). In contrast, sodium valproate potently attenuated spike-and-wave discharges, but neither an additional beneficial effect nor complementary neurochemical effects, were observed in combination with LTG.
I would like to thank my Supervisors Drs. Peter Whitton and Les Fowler for breaking new ground by creating this project, and for their help and advice. Special thanks to my Industrial Supervisor, Dr. Mike Leach, to whom I am indebted for having so generously given me advice, guidance and encouragement. I would also like to express my gratitude to Prof. Norman Bowery, Ex-Departmental Head, for allowing me to freely use equipment which has contributed much to the formation of this work. Thanks also to The Wellcome Foundation (subsequently Glaxo-Wellcome) and the BBSRC for financial support.

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For my parents...
"The great tragedy of science
 - the slaying of a beautiful hypothesis by an ugly fact"

Thomas H. Huxley.
Publications arising from this Thesis

The following publications have resulted from original work presented in this thesis:

Meeting abstracts:


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## ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACh</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>aCSF</td>
<td>Artificial cerebrospinal fluid</td>
</tr>
<tr>
<td>AD</td>
<td>After discharge</td>
</tr>
<tr>
<td>AHP</td>
<td>After hyperpolarisation</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid</td>
</tr>
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<td>AMPH</td>
<td>Amphetamine</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ASP</td>
<td>Aspartate</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood-brain barrier</td>
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<td>BDZ</td>
<td>Benzodiazepine</td>
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<td>BTX</td>
<td>Batrachotoxin</td>
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<td>CBZ</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td>c.f.</td>
<td>Compare with</td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic guanosine 3', 5' monophosphate</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CP</td>
<td>Complex partial</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>C/V</td>
<td>Current/voltage</td>
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<tr>
<td>dB</td>
<td>Decibel</td>
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<td>DMCM</td>
<td>methyl-6,7-dimethoxy-4-ethyl-β-carboline carboxylate</td>
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<tr>
<td>DOI</td>
<td>(±)-2,5-dimethoxy-4-iodoamphetamine</td>
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<td>DOPAC</td>
<td>3,4 dihydroxyphenylacetic acid</td>
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<td>DS</td>
<td>Depolarising shift</td>
</tr>
<tr>
<td>ECD</td>
<td>Electrochemical detector</td>
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<tr>
<td>ECF</td>
<td>Extracellular fluid</td>
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<tr>
<td>ED&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Effective dose (50 %)</td>
</tr>
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<td>Ethylenediaminetetraacetic acid</td>
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<tr>
<td>EEG</td>
<td>Electroencephalograph</td>
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<tr>
<td>EPSP</td>
<td>Excitatory postsynaptic potential</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
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<tr>
<td>GAD</td>
<td>Glutamic acid decarboxylase</td>
</tr>
<tr>
<td>GAERS</td>
<td>Genetic Absence Epilepsy Rats from Strasbourg</td>
</tr>
<tr>
<td>GEPR</td>
<td>Genetic Epilepsy Prone Rat</td>
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### Abbreviations

<table>
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<td>GHB</td>
<td>γ-hydroxybutyrate</td>
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<td>GLN</td>
<td>Glutamine</td>
</tr>
<tr>
<td>GLU</td>
<td>Glutamate</td>
</tr>
<tr>
<td>GTC</td>
<td>Generalised tonic-clonic</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>5-hydroxyindoleacetic acid</td>
</tr>
<tr>
<td>HN</td>
<td>Heated nebuliser</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HPLC-ED</td>
<td>High performance liquid chromatography with electrochemical detection</td>
</tr>
<tr>
<td>HPLC-FD</td>
<td>High performance liquid chromatography with fluorimetric detection</td>
</tr>
<tr>
<td>HPLC-MS</td>
<td>High performance liquid chromatography-mass spectrometry</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine; serotonin</td>
</tr>
<tr>
<td>5-HTP</td>
<td>5-hydroxytryptophan</td>
</tr>
<tr>
<td>HVA</td>
<td>Homovanillic acid</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>INH</td>
<td>Isonicotinic acid hydrazide</td>
</tr>
<tr>
<td>i.p.</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>IPSP</td>
<td>Inhibitory postsynaptic potential</td>
</tr>
<tr>
<td>i.v.</td>
<td>Intravenous</td>
</tr>
<tr>
<td>KCl</td>
<td>Potassium chloride</td>
</tr>
<tr>
<td>LC-TSP-MS</td>
<td>Thermospray liquid chromatography-mass spectrometry</td>
</tr>
<tr>
<td>L-DOPA</td>
<td>3-(3,4-dihydroxyphenyl-2,5,6,d3)-L-alanine</td>
</tr>
<tr>
<td>log P</td>
<td>Partition coefficient</td>
</tr>
<tr>
<td>LTG</td>
<td>Lamotrigine (Lamictal™)</td>
</tr>
<tr>
<td>mA</td>
<td>Milliamps</td>
</tr>
<tr>
<td>MES</td>
<td>Maximal electroshock</td>
</tr>
<tr>
<td>MK-801</td>
<td>(+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d),cyclohepten,5,10-imine maleate</td>
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<tr>
<td>MPTP</td>
<td>1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>mS</td>
<td>Millisecond</td>
</tr>
<tr>
<td>NA</td>
<td>Noradrenaline</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Na VPA</td>
<td>Sodium valproate</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>O.D.</td>
<td>Outer diameter</td>
</tr>
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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>ODS</td>
<td>Octadecylsilicate</td>
</tr>
<tr>
<td>8-OHDPAT</td>
<td>8-hydroxy-2-(di-n-propylamino)-tetralin</td>
</tr>
<tr>
<td>OPA</td>
<td>O-phthaldialdehyde</td>
</tr>
<tr>
<td>PB</td>
<td>Phenobarbital</td>
</tr>
<tr>
<td>PC</td>
<td>Personal computer</td>
</tr>
<tr>
<td>pCPA</td>
<td>O-chlorophenylalanine</td>
</tr>
<tr>
<td>PGE₂</td>
<td>Prostaglandin E₂</td>
</tr>
<tr>
<td>PHN</td>
<td>Phenytoin</td>
</tr>
<tr>
<td>p.o.</td>
<td>Oral</td>
</tr>
<tr>
<td>PTZ</td>
<td>Pentylenetetrazol</td>
</tr>
<tr>
<td>r.p.m.</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>RTN</td>
<td>Nucleus reticularis thalami</td>
</tr>
<tr>
<td>SE</td>
<td>Status epilepticus</td>
</tr>
<tr>
<td>SRF</td>
<td>Sustained repetitive firing</td>
</tr>
<tr>
<td>SRM</td>
<td>Selection reaction monitoring</td>
</tr>
<tr>
<td>SWD</td>
<td>Spike-and-wave discharge</td>
</tr>
<tr>
<td>TAU</td>
<td>Taurine</td>
</tr>
<tr>
<td>t₁/₂</td>
<td>Half-life</td>
</tr>
<tr>
<td>THIP</td>
<td>4,5,6,7-tetrahydroisoazole [5,4-c]pyridin-3-ol hydrochloride</td>
</tr>
<tr>
<td>TTX</td>
<td>Tetrodotoxin</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>VH</td>
<td>Ventral hippocampus</td>
</tr>
<tr>
<td>VLT</td>
<td>Ventrolateral thalamus</td>
</tr>
<tr>
<td>WAG/Rij</td>
<td>Wistar Albino Glaxo/Rijswijk</td>
</tr>
</tbody>
</table>
1.1 Introduction

Epilepsy comes from the Greek "a phenomenon in which man appears to be seized by the forces from within."

With a worldwide prevalence of between 0.5 to 1 %, and an estimated 50 million sufferers, epilepsy is one of the most common neurological disorders to inflict man (Rogawski and Porter, 1990). The primary method of managing patients with epilepsy has been to use chronic administration of anticonvulsant drugs. However, current medical therapy is largely symptomatic, and not always satisfactory. Whilst antiepileptic drugs control seizures in many patients, for others there is no effective prophylactic treatment or easy cure. The need for enhanced anti-epilepsy therapy to allow such patients to lead a normal quality of life, without having to continually live with the fear of a seizure, therefore remains a desperate one.

In October 1991, Lamictal™ (lamotrigine (LTG), 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine; Wellcome Research Laboratories) was successfully marketed in the U.K. as add-on therapy in patients with refractory epilepsy, providing renewed hopes for sufferers.

1.2 Lamotrigine

1.2.1 History

Lamotrigine emerged in the late 1970s from a screening program at The Wellcome Research Laboratories, in which a series of compounds interfering with folate metabolism were evaluated for anticonvulsant activity. The screening was based on the proposal that the antifolate activity of major anticonvulsant drugs, such as phenytoin and phenobarbital, may be relevant for their therapeutic action (Reynolds et al., 1966). Although it is structurally related to antifolate drugs, LTG itself has only weak antifolate activity, and further studies of these compounds failed to show a correlation
between their antifolate and anticonvulsant activities (reviewed by Peck, 1994).

Since those early days, LTG has proved to be an important newcomer to the armamentarium of antiepileptic drugs, and is currently marketed in over 40 countries worldwide including the U.S. (in December, 1994), with approximately 80,000 patient exposures to date (Messenheimer, 1995).

1.2.2 Anticonvulsant clinical efficacy - An overview

Ethical constraints and the possibility of neurogenic injury confines the testing of unproven antiepileptic agents to patients with intractable seizures who are unresponsive to polytherapy. Drugs which display efficacy as add-on therapy in this refractory population may only then be examined as monotherapy (see Appendix for seizure classification).

1.2.2.1 Adjunctive therapy of resistant partial epilepsy with or without secondarily generalised tonic-clonic seizures

LTG has been studied extensively in resistant, partial epilepsy with or without secondarily generalised seizures in randomised double-blind, placebo-controlled trials, eliciting measurable benefits in these previously refractory patients (reviewed by Fitton and Goa, 1995; Leach and Brodie, 1995). Reductions in secondarily generalised tonic-clonic seizures have also been observed (Jawad et al., 1989; Smith et al., 1993).

1.2.2.2 Monotherapy of partial epilepsy and idiopathic generalised tonic-clonic seizures

The recently published results of 4 large scale controlled comparative trials of LTG monotherapy indicate that the drug has a similar efficacy to carbamazepine (CBZ) (Yuen et al., 1994; Brodie et al., 1995; Reunanen et al., 1996) and phenytoin (PHN) (Steiner et al., 1994) against partial onset seizures and idiopathic generalised tonic-clonic seizures, but with a reduced central nervous system (CNS) side-effect profile.
1.2.2.3 Adjunctive therapy of other seizure types

Initial experience in limited numbers of patients suggests a beneficial effect for LTG in idiopathic generalised epilepsy, including typical and atypical absence seizures, atonic seizures, juvenile myoclonic seizures and status epilepticus (reviewed by Fitton and Goa, 1995; Leach and Brodie, 1995). Some efficacy has also been demonstrated in several small studies of patients with Lennox-Gastaut syndrome (Oller et al., 1991; Timmings and Richens, 1992; Oller and Oller-Daurella, 1993).

The findings of all these studies therefore demonstrate adjunctive LTG therapy to have a broad spectrum of anticonvulsant activity, proving to be efficacious for the treatment of previously drug-resistant seizures. Consequently, LTG has established itself as an important new medication for the treatment of epilepsy, and further monotherapy data is required to exclude the possible influence of concomitant antiepileptic agents on the pharmacodynamic effects of LTG itself.

1.3 Epilepsy

Epilepsy is "an occasional and excessive disorderly discharge of nervous tissue"

John Hughlings Jackson (1885).

1.3.1 Diversity of the problem

Epilepsy is not a single disease entity. Rather, the term refers to a group of disorders which have in common the clinical phenomenon of the "seizure."

1.3.2. Classification of epileptic seizures

The various types of epileptic seizures encountered clinically have been classified by the Commission on Classification and Terminology of the International League against Epilepsy, and summarised by Shorvon (1990) and Chadwick (1994), based on
behavioural symptoms and electroencephalographic changes. A summary of the classification of epileptic seizures is shown in the Appendix.

1.3.3 Causes

Epilepsy occurs when the usual balance between excitation and inhibition in the CNS is disturbed. This balance is organised both at a cellular level and a synaptic level.

1.3.3.1 Cellular mechanisms responsible for epileptic events

The following overview has been compiled from general reviews of seizure pathophysiology (Prince, 1985; Dichter and Ayala, 1987; Dichter, 1989; McNamara, 1994; Shin and McNamara, 1994).

1.3.3.1.1 Focal epileptogenesis

Normally, depolarising inward currents such as Na\(^+\) and Ca\(^{2+}\) are balanced by repolarising outward currents such as voltage- and Ca\(^{2+}\)-dependent K\(^+\) currents. During epileptic seizures however, Na\(^+\)- and Ca\(^{2+}\)-dependent excitatory postsynaptic potentials (EPSPs), which are responsible for the depolarisation of neuronal membranes and action potential propagation in the CNS, are amplified. Various mechanisms have been postulated to be responsible for this enhanced synaptic efficacy which include:

- withdrawal of inhibition
- frequency-potentiation of EPSPs
- activation of N-methyl-D-aspartate (NMDA) receptors as the cell depolarises (mediated by a reduction in voltage-dependent Mg\(^{2+}\) blockade)
- potentiation by various neuromodulators (e.g. acetylcholine (ACh), noradrenaline (NA)).

In addition, a number of voltage-dependent intrinsic currents existing in CNS neurones
can supplement the depolarising effects of EPSPs in epileptic foci. These include slowly-inactivating Na\(^+\) and Ca\(^{2+}\) currents and low-threshold, transient L-type Ca\(^{2+}\) currents that are likely to be responsible for Ca\(^{2+}\)-dependent action potentials. Thus, it has been proposed that as the potentiated EPSP depolarises the neurone, a threshold is reached for the development of a slowly inactivating Na\(^+\) current that amplifies the depolarisation. As depolarisation continues, low-threshold Ca\(^{2+}\) currents turn on to further depolarise the neurone, whilst NMDA-mediated excitatory Na\(^+\) and Ca\(^{2+}\) currents are activated, further depolarising the membrane so that it discharges with a burst of action potentials.

The after hyperpolarisation (AHP) that follows this depolarising shift (DS) is responsible for limiting the duration of the depolarisation. Under physiological conditions there are various mechanisms that hyperpolarise neurones:

- short-latency GABA-mediated inhibitory postsynaptic potentials (IPSPs) due to Cl\(^-\) channel opening
- longer-latency, slower IPSPs due to K\(^+\) channel opening
- several types of voltage-sensitive K\(^+\) channels
- Ca\(^{2+}\)-dependent K\(^+\) channels
- Ca\(^{2+}\)-dependent Cl\(^-\) channels

Different cell types have various combinations of these conductances, thus different mechanisms may be responsible for initiating inhibition. Therefore an impairment of these currents will result in the disappearance of the post-DS AHP, being replaced instead by a long and sustained depolarisation with burst firing.

This co-incident decline of inhibition and increase in excitation (both components responsible for normal synaptic regulation of the CNS) thereby allows epileptic activity to spread locally at a distance from the original focus.

Therefore drugs capable of inhibiting sustained repetitive firing (SRF) by blockade of
Na\(^+\) and/or Ca\(^{2+}\) currents and/or potentiating AHP (K\(^+-\)dependent) would be potentially useful in suppressing epileptogenesis.

### 1.3.3.1.2 Generalised non-convulsive epileptogenesis

Studies in genetic absence models suggest that the mechanisms underlying bilaterally synchronous spike-and-wave discharges (SWDs) are different from those of focal epileptogenesis. Low-threshold (T-type) Ca\(^{2+}\) channels, localised in the thalamus, the pontine reticular formation and the anterior olive, seem to play an important role in the genesis of SWDs. In the Genetic Absence Epilepsy Rat from Strasbourg (an experimental model of absences with SWDs), it is considered that the Nucleus Reticularis Thalami (RTN) plays a crucial role in regulating SWDs. This role largely depends on activation of GABAergic cells in the RTN which produce hyperpolarisation, removing inactivation of T-type Ca\(^{2+}\) currents peculiar to RTN neurones, enabling them to produce rhythmic oscillatory activity, that is crucial for SWD generation (reviewed by Snead, 1995).

Thus, drugs capable of blocking T-type Ca\(^{2+}\) currents are potentially useful in suppressing SWDs.

### 1.3.3.2 Neurochemical mechanisms responsible for epileptic events

"When the neurotransmitter substances in the brain are known ... and when the chemical environment of the nerve cell ... are better understood, the neurologist may feel less bewildered by the problem of epilepsy than he is today," Sir Charles Symonds, 1959.

In this section, the neurochemical findings from experimental models of epilepsy and clinical studies are discussed in relation to possible biochemical mechanisms involved in generating and controlling seizure disorders.
1.3.3.2.1 Amino acids

Several amino acids act as putative neurotransmitters in the CNS. Glutamate is however the most abundant excitatory neurotransmitter in the mammalian brain (Fonnum, 1984), and causes increased $\text{Na}^+$ and $\text{K}^+$ permeability leading to membrane depolarisation (Greenmyre et al., 1994). $\gamma$-aminobutyric acid (GABA) in contrast, is the most abundant inhibitory neurotransmitter in the brain, and is responsible for hyperpolarisation of postsynaptic membranes as a result of an increase in $\text{Cl}^-$ permeability (Daily and Jobe, 1986a). Consequently, excitatory (glutamatergic) and inhibitory (GABAergic) neurotransmission in epilepsy has been the focus of extensive research.

1.3.3.2.1.1 Glutamate

The capacity of glutamate or its structural analogues to induce seizures when focally injected in the neocortex or in subcortical nuclei, was first reported by Hayashi in 1954. Since those early findings, various glutamate abnormalities in epilepsy have been discovered.

Genetic and metabolic abnormalities

The possibility that abnormal glutamate metabolism or transport may contribute to epilepsy, has been demonstrated by observations that plasma glutamate levels are elevated in epilepsy. Thus, in a rodent model of epilepsy, plasma glutamate levels were elevated by 35% (EL mice) (Janjua et al., 1992b), with elevations in basal extracellular fluid glutamate concentrations also being observed in kindled rats (Minamoto et al., 1992). Raised plasma glutamate levels have also been demonstrated in patients with generalised epilepsy and their first-degree relatives (Janjua et al., 1992a). These findings have led to the proposal that tissue damage, gliosis and scarring may lead to leakage of glutamate into the extracellular space. This effect together with a reduction in glutamate re-uptake, may lead to accumulation and hence
enhanced levels of excitation and changes in glutamate receptor sensitivity (Bradford, 1995) (see Fig. 1(a)).

Glutamate release

Microdialysis studies have demonstrated an increase in extracellular glutamate concentrations in experimentally-induced seizures in rats, following both tonic-clonic (Rowley et al., 1995b) and kindled seizures (Ueda and Tsuru, 1995). Similarly, in vitro studies of stimulated glutamate release in kindled animals, have also demonstrated enhanced glutamate release (or impaired uptake) in cortex and hippocampus (Leach et al., 1987). However, other studies in animal models, with brief or prolonged seizures, have failed to provide evidence of a change in extracellular glutamate concentrations, even during prolonged convulsions induced by systemic convulsants agents (e.g. picrotoxin) (Millan et al., 1991; Obrenovitch et al., 1996). However, During and Spencer (1993), employing chronically implanted bilateral hippocampal electrodes and microdialysis probes in man, have shown a marked increase in extracellular glutamate concentration before the onset of seizures. Meldrum (1994) has proposed that whilst the events that contribute to an increase in extracellular glutamate before or at seizure onset remain unclear, they may be the consequence of enhanced release or impaired uptake, leading to consequently enhanced activation of postsynaptic glutamate receptors (see Fig. 1(a)).
Fig. 1(a): Glutamate involvement in epilepsy (taken from Bradford, 1995).
A schematic representation of the mechanisms which may contribute to excessive glutamate overflow and consequently mechanisms leading to glutamate-mediated excitatory events, as may occur in epilepsy.

Changes in glutamate receptors

Molecular biological studies have also shown an abnormal expression of, or enhanced functioning in, glutamate receptor subtypes in various epilepsies. Hence, in situ hybridisation studies of glutamate receptor gene expression in rat hippocampus following kainate-induced status epilepticus (SE), have demonstrated an increase in GLUR2 and GLUR3 expression (Friedman et al., 1994). Additionally, autoradiographical studies have demonstrated increases in the density of NMDA and $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainate receptors in human brain (reviewed by Meldrum, 1994).
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Glutamate receptor antagonists

Competitive and non-competitive antagonists acting at postsynaptic NMDA receptors, have been shown to exert anticonvulsant and antiepileptogenic actions in various seizure models, such as audiogenically-induced seizures in mice (Croucher et al., 1982), in photo-sensitive baboons (Meldrum et al., 1983), genetically and epilepsy prone rats (De Sarro and De Sarro, 1992, 1993) and in kindled rats (Croucher and Bradford, 1990).

Glycine site NMDA antagonists as well as competitive AMPA/kainate antagonists, are also effective anticonvulsants in animal seizure models (reviewed by Meldrum, 1994).

Excitatory amino acid-mediated mechanisms are also involved in the modulation and regulation of thalamo-cortical rhythmicity underlying non-convulsive human absence epilepsies. Recent experiments have demonstrated that pharmacological manipulation of NMDA-mediated excitation, results in a profound effect on SWD duration in a number of models of generalised absence seizures. In the γ-hydroxybutyrate (GHB; a GABA metabolite) model of absence seizures (Banerjee and Snead, 1992) and in the GAERS (Marescaux et al., 1992b), administration of either NMDA receptor agonists or antagonists resulted in an attenuation in SWD duration (the reader is referred to Section 6.1 for an explanation of how both agonist and antagonist effects ameliorate such seizures). In the Wistar Albino Glaxo/Rijswijk (WAG/Rij) genetic model of absence seizures, the NMDA receptor antagonist (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine maleate (MK-801; dizocilpine) also reduced SWD duration (Peeters et al., 1989).

1.3.3.2.1.2 Aspartate

Aspartate is capable of acting at some glutamate receptors, and in certain situations is preferentially released at glutamatergic synapses (Meldrum, 1994). Furthermore, Mori and Wada, (1987) have shown aspartate to be epileptogenic, and these findings
have been more recently confirmed in rodent seizure paradigms. Thus, Millan et al. (1993) have demonstrated elevated extracellular concentrations of aspartate in the hippocampus before the onset of pilocarpine-induced limbic seizures (but not during the seizure), whilst Flavin and Seyfried (1994) have shown an elevation in K+-evoked aspartate release from hippocampal slices of epileptic (EL mice) compared to nonepileptic control mice.

1.3.3.2.1.3 GABA

GABAergic systems serve to terminate or limit the spread of seizure activity. Therefore, a failure in GABA systems may also be a possible primary cause of epilepsy (Meldrum, 1989). Indeed, Hayashi (1954) found that topical application of GABA onto canine motor cortex prevented the electroencephalographic spread of focal ictal activity. In addition, drugs which prevent GABA uptake such as tiagabine, are effective anticonvulsant agents (reviewed by Meldrum, 1995), as is sodium valproate (Na VPA), which has been shown to reverse isonicotinic acid hydrazide- (INH) induced seizures and the accompanying reduction in basal extracellular GABA levels, which are raised by Na VPA (Biggs et al., 1994), by possibly augmenting the activity of the GABA synthetic enzyme glutamic acid decarboxylase (GAD) (Phillips and Fowler, 1982). In contrast, drugs capable of inhibiting GAD activity, such as hydrazide (Killam and Bain, 1957) and allylglycine (Horton et al., 1979) are potent convulsants in rodents. Inhibition of the post-synaptic actions of GABA with GABA_A receptor antagonists (Meldrum and Braestrup, 1984) and β-carbolines such as methyl-6,7-dimethoxy-4-ethyl-β-carboline carboxylate (DMCM) (Peterson, 1983), invariably also have a similar proconvulsant action in experimental animals.

Other investigators have suggested a possible failure of GABAergic neurones to be responsible for the aetiology of human epilepsy. In support of this, Ribak (1983, 1985) has reported losses of GABAergic neurones in cortical epileptic foci. Furthermore, a significant decrease in GABAergic neurones in kindled rat amygdala has also been

1 activates muscarinic receptors, which in turn are thought to enhance and maintain the actions of the excitatory amino acids glutamate and aspartate (Olney et al., 1983, 1986).
reported together with associated equivalent losses of GABA (Löschler and Schwark, 1987; Callahan et al., 1991). Recently, Doretto et al. (1994) have also demonstrated K⁺-stimulated GABA release to be significantly reduced in the substantia nigra of genetically epilepsy prone rats (GEPRs), whilst a reduction in extracellular GABA levels in microdialysates from kindled rat amygdala, has also been demonstrated by Kaura et al. (1995). However in humans, immunocytochemistry for GAD has not shown preferential loss of GABAergic interneurones in the hippocampus in anterior temporal lobectomy specimens (Babb et al., 1989), and overall direct evidence for a defect in GABAergic systems in humans, as a major cause of epilepsy, is relatively sparse (Houser, 1991). These observations have led Meldrum (1995) to postulate, that as in the "dormant basket cell" hypothesis for temporal lobe epilepsy (McNamara, 1994), in which recurrent inhibition in the hilus of the dentate gyrus is impaired because of failure of the excitatory input to GABAergic interneurones, reductions in GABA may simply be the result of exaggerated excitatory activity. Hence, by raising the natural levels of synaptic inhibition by GABA-elevating agents, the intrinsic balance of neuronal activity in favour of excitation is overcome. Recently however, During et al. (1995) have demonstrated a reduction in the number of GABA transport proteins in neuronal cell membranes in both human and rat epileptic tissue. These investigators have postulated that a decrease in GABA transporters leads to a reduction in glutamate-induced GABA release. This decrease is likely to occur during reversed GABA transport (i.e. outward) when intracellular Na⁺ levels rise owing to prolonged membrane depolarisation. Such reduced outward GABA transport would lead to reduced inhibition, and subsequently to a dominance of excitation.

In contrast, excessive GABA-mediated inhibition is thought to underlie the genesis of non-convulsive human absence epilepsies (Liu et al., 1991), and raised basal extracellular GABA levels have been reported in GAERS as compared to non-epileptic animals of the same strain (Richards et al., 1995). GABA₄ agonists such as muscimol and 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol hydrochloride (THIP) exacerbate experimental absence seizures, although GABA₄ antagonists do not protect against these seizures (Micheletti et al., 1985). Other GABA-mimetics, such as γ-vinyl-GABA
(vigabatrin), an inhibitor of GABA transaminase, and SKF 89976, a GABA re-uptake inhibitor, also exacerbate absence seizures in GAERS (Marescaux et al., 1992b). The GABA\textsubscript{B} agonist (-)-baclofen increases SWDs in GAERS in a dose-dependent manner (Vergnes et al., 1984), and in contrast to GABA\textsubscript{A} antagonists, selective GABA\textsubscript{B} antagonists (Bittiger et al., 1990; Snead, 1996a), dose-dependently decrease both spontaneous SWDs as well as SWDs potentiated by (-)-baclofen (Marescaux et al., 1992a).

1.3.3.2.1.4 Glutamine

Glutamine is an immediate precursor for neurotransmitter glutamate via the action of glutaminase (Bradford et al., 1978), and an elevation in extracellular glutamine during kindling seizures has been observed by Kaura et al. (1995). Bradford (1995) has proposed that this rise is likely to indicate increased glial synthesis and passive release of glutamine by enhanced glutamate uptake, owing to an increase in glutamate release during the seizure.

1.3.3.2.1.5 Taurine

Taurine is structurally similar to GABA, and reductions have been observed in epileptic foci (Van Gelder, 1982, 1987). These investigators have suggested that losses of taurine from neurones could well be expected to favour the balance of neuronal activity in favour of excitation.

1.3.3.2.2 Monoamines

The monoamines represent another group of neuroactive substances which are capable of regulating the initiation and spread of seizure activity. Their importance in epilepsy was first inferred from studies in which central stores of monoamines were chemically depleted. These treatments resulted in a lowering of pentylenetetrazol (PTZ) seizure thresholds in rodents (Chen et al., 1954), and the abolition of the effects of
amphetamine (AMPH) in raising electroconvulsive seizure thresholds in rabbits (DeSchaepdryver et al., 1962). Later experiments sought to define more clearly the role of individual monoaminergic systems in the control of seizures following chemical manipulations of these systems.

1.3.3.2.2.1 Dopamine

The biochemical evidence for a dopaminergic dysfunction in the brain of epileptic patients is somewhat inconsistent. Some studies report reductions in dopamine (Hiramatsu et al., 1982) and its major metabolite, homovanillic acid (HVA) (Van Woert et al., 1977) in the cerebrospinal fluid (CSF) of idiopathic epilepsies, whilst as much evidence suggests no difference in dopamine release and metabolism in epileptics and age-matched controls sampled from various body fluids (Reynolds et al., 1975; Silverstein and Johnston, 1984; Rao et al., 1989).

Animal models of epilepsy have also produced differing and contradictory evidence for a dopamine dysfunction. Whilst dopamine levels were normal in the parietal cortex of the epileptic beagle (under nonseizing steady-state conditions) (Edmonds et al., 1979), together with dopamine uptake in the epileptic gerbil (Cox and Lomax, 1976), other studies have reported diminished dopamine concentrations. For example, Dailey et al. (1982) have observed a decrease in the subcortical dopamine content, with a reduction in mesencephalic dopamine synthesis also being reported in GEPRs (Laird et al., 1984). Reductions in the levels of striatal dopamine and its metabolites, together with reductions in biochemical markers for dopamine synthesis and release, were also noted by Vriend et al. (1993) for spontaneously epileptic BALB/c mice. However, recent evidence utilising microdialysis to determine extracellular catecholamine levels in the thalamus of spontaneously epileptic rats, have demonstrated a reduction in noradrenaline output but a normal extracellular dopamine concentration (Yan et al., 1993).

Electrically- and chemically-stimulated seizures in animals also show somewhat
differing effects. Thus, increased dialysate dopamine levels were detected in the striatum (McGarvey et al., 1993; Zis et al., 1991) and nucleus accumbens (Nomikos et al., 1991) in response to electroconvulsions. In contrast, sodium pentobarbitone reduced the seizure duration but not the interstitial dopamine concentration (McGarvey et al., 1993), whilst there was also no change in striatal dopamine, HVA or 3,4 dihydroxyphenylacetic acid (DOPAC) efflux in fluorothyl-induced seizures in rodents. In comparison, Cavelheiro et al. (1991, 1994) observed that pilocarpine-induced limbic seizures elevated dopamine levels and lowered dopamine turnover in the hippocampus of rats undergoing status epilepticus or spontaneous seizures. Additionally, Al-Tajir and Starr (1993) detected increased dialysate HVA content in the striatum of rats during pilocarpine-induced seizures. The effects of kindling on dopamine systems is also inconsistent. Kant et al. (1980) reported no change in the K^+-stimulated release of endogenous dopamine from various brain regions in vitro. More recently, Strecker and Monetta (1994) have demonstrated that electrical stimulation of the dorsal hippocampus in rats, kindled to produce stage 3 seizures (represented by forelimb clonus and rearing (Janusz and Kleinrok, 1989)), briefly increased extracellular dopamine and DOPAC levels in the contralateral nucleus accumbens. Other, recent, in situ hybridisation studies of kindled brain tissue, have demonstrated an increase in the mRNA for D_2 receptors, suggesting an up-regulation as a consequence of prolonged seizure activity (Gelbard and Applegate, 1994).

In addition to the evidence provided, an alternative, extensively studied approach to investigating dopaminergic involvement in epilepsy, has been to assess the effects of a selection of dopamine agonists and antagonists for pro- or anticonvulsant activity. Using this approach, it was found that the non-selective dopamine receptor agonist apomorphine, was anticonvulsant against photic seizures induced in the Sengalese baboon *Papio papio* (Meldrum et al., 1975), DBA/2 mouse audiogenic seizures (Anlezark and Meldrum, 1975), and reduced firing of cobalt-induced epileptic foci (Dow et al., 1974). More recently, with the development of specific dopamine receptor agonists and antagonists, the D_1 agonist SKF 38393 was found to be highly proconvulsant in pilocarpine-induced limbic motor seizures (Al-Tajir and Starr, 1990).
Conversely, pretreatment of rats with the D\textsubscript{2} receptor agonist quinpirole, protected against seizures normally induced following a fully convulsant dose of pilocarpine. Furthermore, it is noteworthy that some anticonvulsant drugs may act by augmenting brain dopamine function. CBZ, for example, facilitates the behavioural effects of dopamine agonists (Barros and Leite, 1986). However, the drug has contradictory effects on dopamine metabolism, appearing to reduce dopamine turnover whilst enhancing dopamine cell firing (Waldmeier \textit{et al.}, 1984). CBZ also has a presynaptic dopamine-releasing action both \textit{in vitro} (Barros \textit{et al.}, 1986) and \textit{in vivo} (Kaneko \textit{et al.}, 1993). In contrast, PHN leads to a dopaminergic supersensitivity after prolonged administration to rats (Lepore \textit{et al.}, 1985), and to dopamine-like orofacial dyskinesias in epileptic patients. The broad-spectrum anticonvulsant Na VPA also has a dopamine-enhancing effect (Biggs \textit{et al.}, 1992a), and Zimmer \textit{et al.} (1980) have reported elevated lumbar CSF HVA levels in human volunteers receiving Na VPA medication.

Dopaminergic pathways may also be operative in the control of generalised absence seizures. D\textsubscript{1}/D\textsubscript{2} agonists such as 3-(3,4-dihydroxyphenyl-2,5,6-d\textsubscript{3})-L-alanine (L-DOPA), apomorphine and amphetamine, result in a dose-dependent reduction of SWD duration, whilst D\textsubscript{1}/D\textsubscript{2} antagonists, such as haloperidol and flupentixol, exacerbate experimental absence seizures (Warter \textit{et al.}, 1988; Cools and Peeters, 1992; Depaulis \textit{et al.}, 1994).

The recent comprehensive review by Starr (1996) has led to the conclusion that "many seizures develop more readily and with greater severity in the brains of animals and patients where the intrinsic dopaminergic activity is low," although the precise mechanisms remain to be fully elucidated.

1.3.3.2.2.2 Serotonin

Human and animal studies have also demonstrated an involvement for serotonin (5-hydroxytryptamine; 5-HT) in the aetiology of epilepsy. Kilian and Frey (1973) found that depleting the brain of its 5-HT stores, with the tryptophan hydroxylase inhibitor \textit{p}-chlorophenylalanine (\textit{p}CPA), lowered the threshold for electrically- and chemically-
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induced convulsions in rodents; although a similar action was not evident in the L-
allylglycine-induced model of generalised epilepsy used by Ashton and Waquier
(1981). Conversely, enhancing central synthesis of the monoamine with the 5-HT
precursor, 5-hydroxytryptophan (5-HTP), was found to protect against photically-
induced seizures in *Papio papio* (Meldrum et al., 1972). GEPRs also have reduced
central levels of 5-HT compared to other rodent strains (Dailey and Jobe, 1986b), and
administration of the 5-HT reuptake inhibitor, fluoxetine, significantly reduced
audiogenic seizures in these animals, correlating temporally with enhanced
accumulation of 5-HT in the thalamic extracellular environment (Dailey et al., 1992).
Fluoxetine is also anticonvulsant in rats, in seizures initiated by focal injection of the
GABA<sub>A</sub> antagonist bicuculline into the substantia nigra, indicating the importance of
this structure for the expression of an anticonvulsant effect (Pisani et al., 1992).
Recently, the involvement of specific 5-HT receptor subtypes in the control of seizure
activity, has become clearer. Kindled seizures in a feline model were potently
suppressed by the selective 5-HT<sub>1A</sub> receptor partial agonist 8-hydroxy-2-(di-n-
propylamino)-tetralin (8-OHDPAT) (Wada et al., 1993), whilst the 5-HT<sub>2</sub> receptor
agonist (±)-2,5-dimethoxy-4-iodoamphetamine (DOI) resulted in a potentiation of such
seizures (Wada et al., 1992). Using a range of serotonergic antagonists, Semonova and
Ticku (1992) were able to identify a role for 5-HT<sub>2c</sub>, 5-HT<sub>3</sub>, and 5-HT<sub>4</sub> receptor
subtypes in the DBA/2 mouse audiogenic seizure model. The most consistent findings
from human studies have been the discovery of the likely involvement of central
serotonergic mechanisms in the aetiology of myoclonic epilepsy. Reduced CSF levels
of the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA), have been recorded for
a variety of myoclonic disorders (Deahl and Trimble, 1991), suggesting that central
serotonergic neuronal activity may be compromised in such patients. In post-anoxic
myoclonus, the treating of patients with 5-HTP has been shown to correct CSF 5-
HIAA levels and to reduce myoclonic episodes (Chadwick et al., 1975).

The augmentation of brain 5-HT function has also been implicated in the mechanisms
of action of some anticonvulsant drugs. CBZ, for example in part, has been shown to
produce anticonvulsant effects in GEPRs via an increase in extracellular 5-HT (Yan
et al., 1992), and a depletion of 5-HT by inhibiting its synthesis, significantly decreases the anticonvulsant effectiveness of CBZ (Yan et al., 1992). Similarly, Na VPA also produces a dose-dependent increase in extracellular 5-HT (Biggs et al., 1992a), and Fahn (1978) has reported 5-HIAA levels being increased in the CSF of patients receiving Na VPA treatment for postanoxic myoclonus.

Table 1(a) summarises the actions of the major neurotransmitters implicated in epilepsy.

Table 1(a): Summary of neurotransmitters involved in epilepsy.

<table>
<thead>
<tr>
<th>Neurotransmitter</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cholinergic</strong></td>
<td></td>
</tr>
<tr>
<td>ACh</td>
<td>+</td>
</tr>
<tr>
<td><strong>Aminergic</strong></td>
<td></td>
</tr>
<tr>
<td>Dopamine</td>
<td>±/+</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>-</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>-</td>
</tr>
<tr>
<td>5-HT</td>
<td>±/+</td>
</tr>
<tr>
<td><strong>Amino acid</strong></td>
<td></td>
</tr>
<tr>
<td>GABA</td>
<td>±/+</td>
</tr>
<tr>
<td>Taurine</td>
<td>-</td>
</tr>
<tr>
<td>Glycine</td>
<td>-</td>
</tr>
<tr>
<td>Glutamate</td>
<td>+</td>
</tr>
<tr>
<td>Aspartate</td>
<td>+</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
</tr>
<tr>
<td>Opioid</td>
<td>-</td>
</tr>
</tbody>
</table>

**KEY:** + excitatory  
- inhibitory

This is a list of the principle neurotransmitters thought to be involved in epilepsy, but by no means is exhaustive.
1.3.4 Neuroanatomical structures important in epilepsy

Disturbances within certain brain structures are considered to be specifically responsible for the generation and propagation of seizures. Here, the involvement of those regions pertinent to work presented in this thesis, *i.e.* the hippocampus and thalamus, are reviewed.

1.3.4.1 The hippocampus

The susceptibility of the hippocampus to seizures has long been known (Green, 1964). In particular, seizure activity, occurring in temporal lobe epilepsy, is associated with the hippocampus, as it is too in SE - a condition in which continuous seizure discharges also arise from a characteristic pattern of hippocampal damage. In all models of SE in experimental animals, a loss of GABAergic inhibition has been documented and the resultant disinhibition is likely to be a major cause of the consequent uncontrolled increase in neuronal excitability in SE (McNamara, 1994; Kapur and MacDonald, 1996). Indeed, in juvenile rats, there is evidence to suggest that an overabundance of recurrent excitatory synapses can develop in area CA3 of the hippocampus, leading to excessive and abnormal neuronal discharges and an electrographic spread of seizure activity - such "miswiring" resulting in increased seizure susceptibility in adulthood (Swann *et al.*, 1992).

Consequently, the hippocampal slice preparation has been extensively employed to study the cellular/electrophysiological and neurochemical properties of epileptiform activity. Thus, mimicking seizure-related ionic changes has been shown to induce epileptiform activity in the hippocampus. Application of an artificial cerebrospinal fluid (aCSF) containing elevated levels of $K^+$ and moderately reduced levels of $Mg^{2+}$ and $Ca^{2+}$, elicits interictal-like discharges in the CA3 region, whilst seizure-like events ensue in the CA1 hippocampal area (Traynelis and Dingledine, 1988; Leschinger *et al.*, 1993). Similarly, prolonged electrographic seizures have been recorded in hippocampal slice preparations with electrical stimulation (Rafiq *et al.*, 1993), and by
various pharmacological manipulations (e.g. GABA receptor antagonists: penicillin, bicuculline, picrotoxin) (MacDonald and Olsen, 1994). Furthermore, histological evidence has shown a loss of specific hippocampal neurones in baboons following L-allylglycine-induced seizures (Meldrum et al., 1974). A similar pattern of neuronal loss (confined to the hippocampus) was found in the brains of cats receiving focal septal nuclei injections of ouabain (Baldy-Mouliner et al., 1973). Clinically, hippocampal cellular reorganisation results in a significant control of temporal lobe seizures (De Lanerolle et al., 1992).

1.3.4.1.1 Neurotransmitters and the hippocampus

Numerous neurophysiological, histochemical, biochemical and selective lesion studies have provided evidence for the occurrence of a variety of neurotransmitter systems including glutamate, GABA, noradrenaline and 5-HT, as well as other neuroactive substances such as ACh, in the hippocampus (reviewed by Straughan, 1975).

In the E1 mouse (a genetic variant that exhibits temporal lobe seizures), immunohistochemistry has identified a variety of hippocampal neurochemical abnormalities, involving GABAergic, monoaminergic and peptidergic neurotransmitter systems (King and LaMotte, 1989). More recently, microdialysis has been utilised to demonstrate neurochemical changes in the hippocampus following both electrically- and chemically-induced seizures in rodents. Thus, Zis et al. (1992) reported increased extracellular 5-HT, HVA, choline and ACh levels following acute maximal electroshock (MES) induced either electrically or with fluorothyl. Similarly, an increase in extracellular glutamate together with a decrease in GABA, following MES-induced seizures (Rowley et al., 1995b), and a decrease in basal GABA levels during INH-induced seizures (Biggs et al., 1994) in the ventral hippocampus, have been demonstrated. Conversely, in SE induced by kainic acid, lithium or pilocarpine, there were decreases in glutamate, aspartate and glutamine concentrations and an increase in GABA concentration in the hippocampus (Chapman et al., 1984; Walton, 1993).
So, the hippocampus may play a critical role in initiating seizure discharges resulting from innate neuronal hyperexcitability and possible neurochemical abnormalities associated with hippocampal neurotransmission.

1.3.4.2 The thalamus

It is well-established that the thalamus is a site of neuronal oscillations and that it plays an important role in the regulation of normal thalamocortical rhythmicity (McCormick, 1992; Steriade et al., 1993). Several lines of investigation suggest that the thalamus underlies the SWDs of generalised absence epilepsy. Thus, early studies have shown that electrical stimulation of the thalamus can produce bilaterally synchronous SWDs (Hunter and Jasper, 1949; Pollen et al., 1963). In contrast, lesioning of the ipsilateral thalamus, including the relay nuclei and the reticular nucleus of the thalamus of GAERS, suppressed SWDs on the lesioned side in response to drugs (e.g. PTZ, GHB) which potentiated SWDs in epileptic animals (Vergnes and Marescaux, 1992) Additionally, in PTZ-induced seizures, cortical SWDs were abolished by inhibiting thalamic activity provoked by spreading depression (Pohl and Mares, 1983). Furthermore, intrathalamic injections of GHB into the mediolateral thalamus, but not into the midline thalamus or the reticular nuclei, increased spontaneous SWDs (Liu et al., 1991), demonstrating the involvement of the mediolateral thalamus in particular, in the genesis of spontaneous SWDs in GAERS. More recent evidence has demonstrated the involvement of intrathalamic GABA\(_B\) receptor-mediated neurotransmission in the control of absence seizures (Liu et al., 1992). These investigators have shown that (-)-baclofen, when injected into the specific relay nuclei and the reticular nuclei of the thalamus, increased spontaneous SWDs in GAERS, whilst CGP 35348 (GABA\(_B\) receptor antagonist) decreased seizures. Neither treatment was effective when administered into the midline thalamus. Furthermore, an increase in the number of thalamic GABA\(_B\) receptors has been reported in the lethargic (lh/lh) mouse model of absence epilepsy (Lin et al., 1993), as has an elevation in thalamic basal extracellular GABA levels (compared to non-epileptic rats of the same strain (Richards et al., 1995).
1.3.4.2.1 Neurotransmitters and the thalamus

GABA_B receptor-mediated neurotransmission has been extensively shown to play a major role in the control of SWDs (reviewed by Marescaux, 1992a; Snead, 1995), hence GABA_B receptor antagonists are effective anti-absence agents, whilst GABA_A receptor agonists exacerbate such seizures.

Since modulation and regulation of thalamocortical rhythmicity also occurs through glutamate-mediated recurrent excitation between thalamocortical and corticothalamic pathways, glutamate receptor antagonists have also been demonstrated to reduce absence seizures. In support of these findings, recent evidence has shown a significant increase in strychnine-insensitive ³H glycine binding in layers IV to VI of the parietal cortex in GAERS (Snead et al., 1992), and an enhanced expression of NMDA-mediated excitation in the middle and deep cortical layers of GAERS (Pumain et al., 1992).

Enhanced monoaminergic (noradrenaline and dopamine) and cholinergic inputs into the thalamocortical circuitry also exacerbate absence seizures (reviewed by Snead, 1995).

Excessive intrinsic (GABA_B receptor-mediated) and/or extrinsic (glutamatergic, monoaminergic, cholinergic) inputs into the thalamus are then, capable of disturbing the fine balance between excitation and inhibition, and of generating SWDs characteristic of non-convulsive epilepsies.
1.4 The role of ion channels in the mechanism of action of anticonvulsant drugs

Various experimental findings have demonstrated that modulation of Na\(^+\), K\(^+\) and Ca\(^{2+}\) channels is an important action of many well-established and new anticonvulsant agents.

1.4.1 Na\(^+\) channels

Several well-established antiepileptic drugs, such as PHN and CBZ, are thought to act by inhibition of voltage-dependent Na\(^+\) channels that mediate action potential generation (Rogawski and Porter, 1990). The block of Na\(^+\) channels by PHN and CBZ is voltage- and frequency-dependent, and may result from the preferential binding of these drugs to the inactivated state of the channel, which greatly reduces the probability of channel opening (Catterall, 1987; Faingold, 1992). These effects of PHN and CBZ allow them to inhibit neuronal firing under abnormal conditions of neuronal excitation, whilst having little effect under normal conditions (Catterall, 1987). Additionally, this mechanism of PHN and CBZ is considered to underlie the inhibition of SRF of isolated neurones (Matsuki et al., 1984; McLean and MacDonald, 1986). Na VPA is also capable of inhibiting SRF at therapeutically relevant concentrations (McLean and MacDonald, 1986). Furthermore, at concentrations achieved in the treatment of SE, a reduction of SRF was also achieved with phenobarbital (PB) and benzodiazepines (BDZs). Inhibition of SRF is widely considered to be in part, the basis for anticonvulsant drug action against generalised tonic-clonic seizures. More recently, several newly developed anticonvulsant drugs, including oxcarbazepine, topiramate, zonisamide, remacemide and ralitoline, are thought to act, at least in part, by inhibition of voltage-dependent Na\(^+\) channels in a similar manner to PHN and CBZ (Rogawski and Porter, 1990) (see Fig. 1(b)).
Fig. 1(b): Antiepileptic drugs capable of Na⁺ channel modulation.
A schematic representation of the mechanism of action of antiepileptic drugs which inhibit voltage-dependent Na⁺ channels and consequently may be capable of inhibiting the pathological release of excitatory neurotransmitters such as glutamate.
1.4.2 K⁺ channels

Since K⁺ conductances are involved in the control of neuronal excitability, activation of K⁺ channels, provides an alternative approach to Na⁺ channel blockade for reducing neuronal excitability. Several anticonvulsant agents, such as cromakalim, have been reported to affect K⁺ channels, but mostly only at supratherapeutic concentrations, and when directly applied into the brain, since they are unable to penetrate the blood brain barrier (Faingold, 1992).

1.4.3 Ca²⁺ channels

In epileptic events, Ca²⁺ influx leads to the development of the DS which underlies epileptic activity, and furthermore, T-type Ca²⁺ channel activation in thalamic neurones, is responsible for the generation of SWDs characteristic of absence seizures. Several clinically established antiepileptic drugs are thought to interact with voltage-gated Ca²⁺ channels. Thus PHN and CBZ, barbiturates and BDZs have all been observed to reduce Ca²⁺ influx into synaptic terminals, although in some cases above clinically effective concentrations (Faingold, 1992). Barbiturates block L-type (long-lasting) Ca²⁺ channels and N-type (neuronal) Ca²⁺ channels but not T-type (transient) Ca²⁺ channels. N-type Ca²⁺ channels are involved in presynaptic neurotransmitter release, therefore such drugs are capable of reducing neurotransmitter release. PHN suppresses N- and T-type Ca²⁺ currents, whilst ethosuximide, trimethadione and Na VPA use-dependently block T-type Ca²⁺ channels in thalamic neurones, this effect likely to be responsible, in part, for their antiabsence effects (Zhang et al., 1996a, 1996b).
1.5 Actions of lamotrigine

1.5.1 Effects in Animal seizure models

For an explanation of animal seizure paradigms the review by Fischer (1989) is recommended.

1.5.1.1 Maximal electroshock-induced seizures

LTG dose-dependently blocked hindlimb extension in mice and rats (Miller et al., 1986) (see Table 1(b)).

1.5.1.2 Pentylenetetrazol-induced seizures

Both hindlimb extension following PTZ-induced seizures in rats and mice (Miller et al., 1986) and sound-induced clonic seizures in GEPRs (Smith et al., 1993) were suppressed by LTG, but clonus latency was not increased in the former (see Table 1(b)).

1.5.1.3 Limbic system after-discharges

LTG reduced hippocampal after-discharge duration in the rat and dog, as well as cortical after-discharges in the marmoset (Wheatley and Miller, 1989) (see Table 1(b)).

1.5.1.4 Electrically-induced kindled seizures

LTG did not block the development of kindling by electrical stimulation of the frontal cortex, but did significantly reduce the number of kindled seizures, as well as the after-discharge duration (O’ Donnell and Miller, 1991). More recently, Otsuki et al. (1995), have shown LTG pretreatment to significantly suppress both amygdaloid and hippocampal kindled seizures, and also the after-discharge duration of kindled seizures (see Table 1(b)).
<table>
<thead>
<tr>
<th>Convulsant agent &amp; stimulus intensity</th>
<th>Seizure type</th>
<th>Species</th>
<th>Route of administration</th>
<th>ED$_{50}$ (mg/kg)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MES (25 mA)</td>
<td>generalised</td>
<td>mouse</td>
<td>p.o.</td>
<td>2.60</td>
<td>Miller et al., 1986</td>
</tr>
<tr>
<td>(250 mA)</td>
<td>tonic-clonic (GTC)</td>
<td>rat</td>
<td>p.o.</td>
<td>1.90</td>
<td>Miller et al., 1986</td>
</tr>
<tr>
<td>PTZ (10 mg/ml)</td>
<td>GTC</td>
<td>mouse</td>
<td>p.o.</td>
<td>7.20</td>
<td>Miller et al., 1986</td>
</tr>
<tr>
<td>GEPRs ( audiogenic)</td>
<td>GTC</td>
<td>rat</td>
<td>i.p.</td>
<td>1.80</td>
<td>Smith et al., 1993</td>
</tr>
<tr>
<td>(10-12 KHz, 60 s, 100-120 dB)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrically-evoked hippocampal AD</td>
<td>complex-</td>
<td>rat</td>
<td>i.v.</td>
<td>11.70</td>
<td>Wheatley and Miller, 1989</td>
</tr>
<tr>
<td>(20 Hz, 2 ms, 5 s)</td>
<td>partial (CP)</td>
<td>dog</td>
<td>i.v.</td>
<td>4.50</td>
<td>Wheatley and Miller, 1989</td>
</tr>
<tr>
<td>Electrically-evoked cortical AD</td>
<td>CP</td>
<td>marmoset</td>
<td>i.v.</td>
<td>5.0 - 15.0</td>
<td>Wheatley and Miller, 1989</td>
</tr>
<tr>
<td>(20 Hz, 2 ms, 5 s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrically-induced Kindling</td>
<td>CP</td>
<td>rat</td>
<td>p.o.</td>
<td>3.0 - 18.0</td>
<td>O'Donnell and Miller, 1991</td>
</tr>
<tr>
<td>(60 Hz, 1 ms, 1 s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slow PTZ infusion (10 mg/kg)</td>
<td>absence</td>
<td>mouse</td>
<td>i.p.</td>
<td>5.0 - 40.0</td>
<td>Baxter et al., 1995</td>
</tr>
</tbody>
</table>

Table 1(b): Summary of the anticonvulsant efficacy of lamotrigine in a variety of animal seizure paradigms.
1.5.2 Effects on biochemical systems

1.5.2.1 Radioligand binding profile

LTG, at concentrations up to $10^{-4}$ M, did not inhibit adenosine ($A_1$ and $A_2$), GABAergic, adrenergic ($\alpha_1$, $\alpha_2$, $\beta$), dopaminergic ($D_1$, $D_2$), histamine ($H_1$), muscarinic ($M_1$, $M_2$) or serotonin ($5-HT_2$) radioligand binding to rat brain membranes. However, LTG did inhibit binding to $5-HT_3$ receptors ($pKi = 4.75$), although the significance of this effect remains unclear (Meldrum and Leach, 1994; Leach et al., 1995). Furthermore, LTG, at concentrations up to 100 $\mu$M, also had no effect on $^3$H-saxitoxin (sodium channel site 1) binding or $^{45}$Ca$^{2+}$ channel ($^4$H-nitrendipine and dihydropyridine) binding sites. $^3$H-batrachotoxinin (sodium channel site 2) binding was however blocked, as was veratrine-induced uptake of $^{14}$C-guanidine into rat cerebral cortex synaptosomes (Riddall et al., 1993).

1.5.2.2 Effects on neurotransmitter release

LTG does not alter neurotransmitter release from rat brain slices evoked by high $K^+$, but does block veratrine-induced glutamate and aspartate release, and at higher concentrations GABA and ACh release (Leach et al., 1986). More recently, Teoh et al. (1995) have also reported a preferential inhibition of electrically-evoked glutamate release from rat isolated spinal cord slices and less potently that of GABA. Other investigators have demonstrated that by inhibiting the release of glutamate, LTG inhibits the production of nitric oxide (Lizasoain et al., 1995). Smith et al. (cited in Meldrum and Leach, 1994) have provided the only known in vivo neurochemical effects of LTG to date, demonstrating LTG to reduce the increase in extracellular glutamate and other amino acids during focal ischaemia in the rat.

1.5.2.3 Effects of LTG on NMDA receptors

Leach et al. (1995) have reported LTG to be ineffective at inhibiting both NMDA-
evoked depolarisations and NMDA-evoked cGMP formation in rat brain slices. In addition LTG does not exhibit NMDA receptor antagonist activity in behavioural (Leach et al., 1991) or electrophysiological (Lees and Leach, 1993) studies.

1.5.2.4 Effects on excitotoxicity

McGeer and Zhu (1990) studied the effects of LTG on kainate, quinolinate and ibotenate lesions induced in the rat striatum. LTG decreased the excitotoxic effects of kainate but not quinolinate or ibotenate. The pathological action of kainate is dependent on synaptic glutamate release, further suggesting that the neuroprotective effects of LTG may relate to its effects on glutamate release.

1.5.3 Electrophysiological effects

LTG is capable of inhibiting SRF by blockade of type IIa Na⁺ channels in cultured neurones and cells in a use- and voltage-dependent manner, without affecting the resting membrane potential (Lang and Wang, 1991; Cheung et al., 1992; Lees and Leach, 1993; Xie et al., 1995). Since SRF is dependent on the prolongation of the inactivation of voltage-dependent Na⁺ channels, Lees and Leach (1993) have shown that LTG by blocking SRF, suppresses the release of glutamate and consequently glutamate-evoked bursts of action potentials.

The effects of LTG on Ca²⁺ currents remain unclear. Whilst Lang et al. (1993) have shown LTG to be ineffective at inhibiting L- and T-type Ca²⁺ currents in rat pituitary cells, at concentrations effective at blocking Na⁺ conductances, Lees and Leach (1993) have reported LTG to inhibit a "presumptive" Ca²⁺ current in rat cortical slices. However, more recent evidence has demonstrated LTG to inhibit both N- and P-type Ca²⁺ currents in cortical neurones, also at concentrations which effectively inhibited Na⁺ currents (Stefani et al., 1996).
1.5.4 Other effects

1.5.4.1 Antiparkinsonian effects

Based on the proposal that blockade of glutamatergic transmission is the mechanism of action of some established antiparkinsonian drugs (e.g. memantine and amantadine (Kornhuber et al., 1989, 1991)), LTG was tested for potential antiparkinsonian activity in patients with Parkinson's disease. Zipp et al. (1993) reported four out of five patients to improve on LTG treatment in an early study, but have failed to confirm this antiparkinsonian effect in a subsequent placebo controlled trial of 20 patients with Parkinson's disease (Zipp et al., 1995). Animal models of Parkinsonian activity have also demonstrated conflicting effects mediated by LTG. Thus, Jones-Humbles et al. (1994) reported 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopamine depletion to be significantly less in mice pretreated with LTG. In contrast, Löschman et al. (1995), utilising various models of Parkinson's disease, failed to observe a restoration in locomotor activity following LTG pretreatment.

1.5.4.2 Cognitive effects

In the T-maze test of working memory, LTG does not impair the working memory of rats. Similarly, in rats trained to discriminate phencyclidine from saline, LTG induced only saline appropriate responses (Leach et al., 1991). More recently, LTG has been tested in a novel operant learning paradigm, in which it produced some impairment in learning performance (Hudzik and Palmer, 1995).

1.5.4.3 Anxiolytic effects

Critchley (1994) evaluated the anxiolytic properties of LTG in the Vögel test of anxiety, based on the premise that glutamate receptor antagonists (i.e. NMDA receptor) have been shown to be anxiolytic. LTG dose-dependently increased punished drinking responses, suggesting that LTG may be anxiolytic.
1.5.4.4 Analgesic effects

LTG has been demonstrated to inhibit the development of PGE$_2$-induced sustained hyperalgesia, as well as blocking the refractory effect of the flexor digitorum longus muscle to noxious stimuli (Nakamura-Craig and Follenfant, 1992a). LTG is also analgesic in a diabetic model of neuropathic pain (Nakamura-Craig and Follenfant, 1992b).

1.5.4.5 Cerebroprotective effects

Glutamate is widely considered to be responsible for producing the neurotoxic effects associated with cerebral ischaemia (Rothman and Olney, 1986; Meldrum and Garthwaite, 1990). Consequently, LTG has been tested in numerous models of cerebral ischaemia. In both animal models of global and focal ischaemia (Rataud et al., 1994; Smith and Meldrum, 1995; Wiard et al., 1995), LTG has exhibited neuroprotective properties. In the latter study, LTG was also shown to reduce ischaemia-induced glutamate and aspartate release.

1.5.4.6 Effects on drug withdrawal

Excitatory amino acids have been implicated in producing in part, the symptoms and signs of opiate abstinence (Lizasoain et al., 1996). Lizasoain et al. (1996) have recently demonstrated that LTG reduced the behavioural and motor symptoms associated with morphine withdrawal in mice.

The consistent findings of these studies suggest that LTG may act primarily by Na$^+$ channel blockade consequently inhibiting the pathological release of glutamate, as may occur in epilepsy (Leach et al., 1995) (see Fig. 1(c)).
(a) Excessive glutamate release

(b) Proposed action of lamotrigine

Fig. 1(c): Simplified proposed mechanism of action of lamotrigine (adapted from Peck, 1992).

Excessive glutamate release is implicated in the pathophysiology of epilepsy. LTG is considered to inhibit voltage-dependent Na' channels and excessive glutamate release.
Chapter 1: General Introduction

1.6 AIMS

In the preceding sections of this Introduction, important experimental and clinical evidence regarding the involvement of central excitatory and inhibitory neurotransmission in the pathophysiologies of epilepsies has been presented, together with a brief review of the known pharmacodynamic actions of the novel antiepileptic drug LTG upon these systems.

Whilst in vitro models such as brain slices and synaptosomal preparations have been extensively utilised in the past to study the action of drugs on neurotransmitter release and the presynaptic regulation of release, it is impossible to recreate the environment of the nervous tissue in an in vitro situation. Neither is it possible to relate the release of a specific, endogenous neurotransmitter with either a physiological or behavioural event. Therefore, given the lack of currently available data regarding the effects of LTG on central neurochemistry in vivo, intracerebral microdialysis and high performance liquid chromatography (HPLC) have been utilised to study the neurochemical effects of LTG in normal animals, and further correlated with electroencephalographic (EEG) changes in epileptic rats, using various pharmacological manipulations.

1.6.1 Principles of microdialysis

A fine dialysis fibre (200-600 μm) is implanted into a selected brain area. Low molecular weight compounds diffuse down their concentration gradients from the brain extracellular fluid (ECF) into an artificial cerebrospinal fluid (a CSF) that flows through the fibre at a constant rate, and can be analysed using highly sensitive assay procedures (e.g. HPLC). This method thereby allows manipulation of the extracellular environment by drugs which may enter the CNS by any route (either infused through the probe or systemically administered) and measuring the subsequent neurochemical changes.
Microdialysis has consequently become an established method for monitoring the extracellular concentrations of a variety of endogenous and exogenous substances. The brain is particularly amenable to study using this technique (reviewed by Benveniste, 1989), and is now often a method of choice for in vivo monitoring of the extracellular space. The technique has a number of advantages over other systems used for studying the brain extracellular environment and these are summarised in Table 1(c). Detailed information regarding microdialysis procedures is provided in Chapter 2. As indicated in sections 1.2.4.1 and 1.2.4.2, the hippocampus and thalamus are considered to play crucial roles in the generation and spread of seizure activity. In addition, both structures are richly innervated by monoaminergic and amino acid neurotransmitter pathways, allowing the collection and analysis of a large number of neurotransmitters and metabolites, using the microdialysis technique. Since many of these neuroactive substances share roles in controlling neuronal excitability via interaction with receptors, activation or blocking of which can profoundly alter the character, duration and intensity of seizures, the neurochemical effects of LTG were investigated in these structures. Furthermore methodologically, both the hippocampus and thalamus in the rat lend themselves to microdialysis, allowing microdialysis probes to be implanted into these structures with relative ease. In all microdialysis experiments presented in successive chapters, conscious animals were employed, thereby overcoming the problems associated with anaesthesia-induced neurochemical changes, such as the compromising of neuronal function and possible interactions with test drugs which may affect neurotransmitter efflux (Marsden, 1985).
Chapter I: General Introduction

Table 1(c): Advantages and disadvantages of various *in vivo* techniques.

<table>
<thead>
<tr>
<th></th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In situ</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ion-selective microelectrode</td>
<td>a. Examination of all brain regions</td>
<td>a. Only detects ions</td>
</tr>
<tr>
<td></td>
<td>b. Time resolution &lt; 1 s</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c. Tip of electrode &lt; 4 µm</td>
<td></td>
</tr>
<tr>
<td>Carbon fibre microelectrode</td>
<td>a. Examination of all brain regions</td>
<td>a. Only detects oxidisable compounds</td>
</tr>
<tr>
<td></td>
<td>b. Use in conscious animals</td>
<td>b. Selectivity poor without previous HPLC analysis</td>
</tr>
<tr>
<td></td>
<td>c. Time resolution &lt; 1 min.</td>
<td>c. Drainage</td>
</tr>
<tr>
<td></td>
<td>d. Tip of electrode &lt; 300 µm</td>
<td>d. Some electrodes have a short working life <em>in vivo</em></td>
</tr>
<tr>
<td><strong>Ex situ</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortical cup</td>
<td>a. No tissue penetration</td>
<td>a. Time resolution &gt; 10 min.</td>
</tr>
<tr>
<td></td>
<td>b. Use in conscious animals</td>
<td>b. Only cortex can be analysed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c. Drainage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d. Deproteinisation before HPLC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>e. Enzymatic degradation of compounds</td>
</tr>
<tr>
<td>Push-pull cannula</td>
<td>a. Examination of all brain regions</td>
<td>a. Time resolution &gt; 10 min.</td>
</tr>
<tr>
<td></td>
<td>b. Use in conscious animals</td>
<td>b. Drainage</td>
</tr>
<tr>
<td></td>
<td>c. BBB(^1) intact following implantation</td>
<td>c. Deproteinisation before HPLC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d. Enzymatic degradation of compounds</td>
</tr>
<tr>
<td></td>
<td></td>
<td>e. Tissue trauma following implantation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>f. Cannula size &gt; 1 mm</td>
</tr>
<tr>
<td>Microdialysis probe</td>
<td>a. Examination of all brain regions</td>
<td>a. Time resolution &gt; 5 min.</td>
</tr>
<tr>
<td></td>
<td>b. Use in conscious animals</td>
<td>b. Drainage</td>
</tr>
<tr>
<td></td>
<td>c. BBB intact following implantation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>d. Probe diameter &lt; 600 µm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>e. Minute tissue trauma within the first 48 hrs.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>f. No need for deproteinisation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>g. No enzymatic degradation</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Blood brain barrier (Modified from Benveniste, 1989).
The studies performed in this thesis can be divided into three categories:

a) Effects of acute and chronic LTG treatment on central neurochemistry - relevance to clinical anticonvulsant efficacy

b) Comparison of the neurochemical effects of LTG with conventional antiepileptic drugs with similar mechanisms of action

c) Neurochemical and electroencephalographic effects of LTG in epileptic rats

All these effects are discussed with relevance to the anticonvulsant mechanism(s) of action of LTG.
2.1 Animals and Husbandry

Male albino Wistar rats (250-300 g; Bantin & Kingman Ltd., Hull, U.K.) and Genetic Absence Epilepsy Rats from Strasbourg (GAERS) (225-310 g; home-bred from an original breeding stock from Strasbourg) were group-housed for at least 7 days under conditions of constant humidity (40-60%) and temperature (20 ± 1 °C) prior to usage. Animals were conditioned to a 12 h light-dark cycle, with lights on from 07.00 to 19.00 hrs each day. Access to food (Rat & Mouse maintenance diet, Bantin & Kingman Ltd.) and water was ad libitum.

2.2 The Microdialysis Experiment

2.2.1 The Microdialysis probe

Concentric microdialysis probes (see Fig. 2(a) for schematic diagram) were constructed in the laboratory as outlined below:

(a) Two pieces of fused silica capillary tubing (Scientific Glass Engineering, Milton Keynes), 2 and 3 cms in length, were inserted into a stainless steel tube (24 G, 1.5 cm length, 0.52 mm O.D.; Tomlinson Tube & Instrument Ltd., Warwicks.), which represented the shaft of the probe, such that the longer piece protruded 1 cm through the end of the shaft (= inlet cannula), whilst the shorter extended 1 cm down into the shaft (= outlet cannula). Both pieces of fused silica tubing were secured at the proximal end of the shaft with a drop of araldite rapid-setting epoxy resin (Ciba-Geigy Plastics Ltd.).

(b) Two pieces of non-sterile, fine-bore polyethylene tubing (pp10 tubing, 0.28 mm I.D., 0.61 mm O.D.; Portex Ltd., U.K.), 9 and 7 cms in length, which formed the inlet and outlet of the probe, were then fitted. The longer piece was passed over the exposed proximal end of the inlet cannula, whilst the shorter piece was passed over the outlet cannula which had been trimmed down to 0.5 cm. These were then secured with epoxy resin adhesive.
Chapter 2: Materials and methods

Fig. 2(a): A schematic cross-sectional representation of a concentric microdialysis probe (not drawn to scale).
Arrows depict direction of flow.
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(c) The length of the tip of the inlet cannula protruding from the distal end of the probe shaft, could be varied depending on the size of the brain structure under investigation. Once this had been trimmed to the appropriate length (4 mm - ventral hippocampus and 2 mm - ventrolateral thalamus), with the aid of a binocular microscope, dialysis membrane (cuprophan capillary membrane, type F1 8 200, 0.2 mm O.D., 10 KD molecular weight cut-off; Gambro, GFE9 dialysis membrane, Hechingen, Germany) was slipped over the inlet cannula, and pushed up into the shaft until resistance was met. The tip of the membrane was then sealed with glue as was the other end where it entered the shaft of the probe.

This design allowed the infusing liquid to enter through the proximal end of the inlet cannula, and flow distally all the way to its end, where it changed direction and returned in the space between the inlet cannula and the membrane, where the microdialysis took place.

Prior to implantation, all probes were slowly infused with an isotonic, physiological buffer, which will be referred to as artificial cerebrospinal fluid (aCSF) (composition: 125 mM NaCl, 2.5 mM KCl, 1.18 mM MgCl₂, 1.26 mM CaCl₂; pH 7.4 with 0.2 M phosphate buffer), to test for functional integrity. If leaks were evident, or an unusually high resistance to flow was present, probes were discarded. This procedure was completed by flushing with de-ionised water to prevent salt crystallisation.

2.2.2 In vitro Recoveries of Microdialysis Probes

The effectiveness of the constructed dialysis probes to recover known concentrations of an analyte was determined in vitro, to control the degree of experimental error that could be introduced into an experiment due to variations in recovery from probe to probe (may be due to differences in probe construction e.g. more or less epoxy on the dialysis membrane).

In vitro recoveries for both amino acids and monoamines were determined by placing
probes into standard solutions of the appropriate neurochemicals. Dialysis probes were suspended into a bathing medium composed of 1 μM 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) for recoveries of monoamines and their metabolites, or 1μM L-aspartic acid (ASP), L-glutamic acid (GLU), L-glutamine (GLN), taurine (TAU) and γ-aminobutyric acid (GABA) for amino acid recoveries (all compounds were obtained from Sigma, U.K.). All substances were dissolved in aCSF. The vessel containing the bathing medium was in turn suspended in a slowly-shaking water bath maintained at 37 °C, to mimic in vivo conditions.

To determine whether the use of an iso-osmotic infusion solution altered the uptake characteristics of the probe, pulses of aCSF made hypertonic by increasing the K⁺ (to 100 mM) concentration, were delivered via the probes for a 30 min. period. Similarly, the effects of 50 μM veratridine upon probe recoveries were evaluated (both elevated K⁺ and veratridine, infused through the microdialysis probe, have been used in subsequent chapters).

Dialysis probes (n=4) were connected to a micro-infusion pump (Harvard Apparatus, syringe infusion pump 22; U.S.A.) and infused with aCSF at a flow rate of 0.5 μl/min. ACSF was delivered to the probe inlets via a 40 cm length of pp1O tubing (which had been flushed with aCSF to prevent delay in the introduction of aCSF to the probe) fitted to a gas-tight 500 μl Hamilton microsyringe. Following a 60 min. stabilisation period, four 30 minute samples were collected. These samples, together with an equivalent volume of the standard bathing medium in the absence and presence of elevated K⁺ (100 mM) or veratridine (50 μM) (which may interfere with the neurochemicals being studied), were analysed by high performance liquid chromatography (HPLC) with electrochemical or fluorimetric detection for monoamines or amino acids respectively.

From these recovery experiments, the % relative recovery of analytes from the bathing
medium was calculated as follows:

(Reproduced from Benveniste, 1989)

\[
\text{Recovery in vitro} = \frac{C_{\text{out}}}{C_{\text{in}}}
\]

where \( C_{\text{out}} \) is the analyte concentration in the outflow and \( C_{\text{in}} \) is the analyte concentration in the bathing medium. Accepting that conditions in vitro can only approximate to those in vivo, estimates of actual brain extracellular levels of monoamines and amino acids were made using:

\[
C_{\text{in}} = \frac{C_{\text{out}}}{\text{recovery in vitro}}
\]

where \( C_{\text{in}} \) is the extracellular concentration and \( C_{\text{out}} \) is the analyte concentration in the probe outflow in vivo.

(a) \hspace{1cm} (b)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>% Recovery</th>
<th>Analyte</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOPAMINE</td>
<td>40.8 ± 4.0</td>
<td>ASP</td>
<td>39.9 ± 2.0</td>
</tr>
<tr>
<td>DOPAC</td>
<td>26.7 ± 6.3</td>
<td>GLU</td>
<td>37.2 ± 2.0</td>
</tr>
<tr>
<td>HVA</td>
<td>18.6 ± 3.8</td>
<td>GLN</td>
<td>37.4 ± 3.0</td>
</tr>
<tr>
<td>5-HT</td>
<td>25.7 ± 7.1</td>
<td>TAU</td>
<td>50.7 ± 3.5</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>19.2 ± 1.9</td>
<td>GABA</td>
<td>47.9 ± 4.2</td>
</tr>
</tbody>
</table>

Table 2 (a): \textit{In vitro} relative recoveries of (a) monoamines and (b) amino acids from mixed standard solutions.

All experiments were performed at a flow rate of 0.5 \( \mu \text{l/min.} \) and 37 °C; Values are means ± s.e.mean (n=4).

'\text{The presence of elevated K'}^+ {100 mM} \text{ or veratridine (50 \( \mu \text{M} \)) in the aCSF did not alter probe recoveries.'
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The material that dialysis membranes are made off, apart from allowing the passage of water and small solutes may also interact with some of these solutes, and thus the presence of surface charges may explain, for example, the lower recoveries of acidic amino acids as compared to neutral ones.

Dialysate data in subsequent chapters has not been corrected for *in vitro* recoveries, since there are several types of problem with attempting to extrapolate from *in vitro* to *in vivo* conditions (Morrison *et al.*, 1991):
(a) *In vitro* it is impossible to mimic *in vivo* metabolism
(b) Microvascular exchange of substances is impossible to achieve *in vitro*
(c) The diffusion of substances through the extracellular fluid, a property related to diffusion through the brain parenchyma, as well as to active elimination from the tissue by uptake into cells and blood capillaries, cannot occur *in vitro*
(d) The tortuosity of the brain parenchyma will alter the speed of diffusion as compared to that through water or artificial cerebrospinal fluid
(e) Convection in the *in vitro* bath is uncontrolled and uncharacterised, therefore having an unknown effect on extraction. As a result, the extraction and transient concentration time scales can be very different in *in vitro* and *in vivo* situations.

However, probes with unusually low recoveries were not used in microdialysis experiments.

### 2.2.3 Microdialysis Probe implantation

Rats were anaesthetised with chloral hydrate (400 mg/kg i.p.; dissolved in 0.9 % saline) and secured in a stereotaxic frame with blunt ear bars (David Kopf, U.S.A.), and with the incisor bar set according to the size of the animal, ensuring that the skull surface was flat. All animal procedures were performed in strict accordance with the guidelines stated in the Animals (Scientific Procedures) Act, 1986. A medial incision of the skin overlying the skull was then made, and the skin and muscles retracted to expose the skull surface. Any bleeding was controlled with cotton wool. Having
exposed the skull bone sutures, bregma was located and carefully marked with pencil. At this point, stereotaxic co-ordinates derived from the stereotaxic atlas of the rat brain by Paxinos & Watson (1986), were used to position a dental drill (tungsten carbide burr tip, 2 mm), held by a micro-manipulator, above the skull surface dependent on the brain area under investigation. Co-ordinates for the areas studied during the course of this research are given in Table 2(b). A burr hole was carefully drilled through the skull at the appropriate co-ordinates and the dura mater exposed. This was then removed with the tip of a sterile hypodermic needle before probe insertion. Two additional burr holes, not quite penetrating through the skull, were drilled in positions on the skull distant from the initial hole, and small steel screws (size 10B, Clarkenwell Screw Company, Holborn, London) were inserted and tightened, so that approximately one half of the shaft was embedded in the skull, with a jeweller's screw driver. Typically, one screw would be placed anterior and to the left of bregma, whilst the other would be placed posterior and to the right of bregma.

<table>
<thead>
<tr>
<th>Structure</th>
<th>'Anterior'</th>
<th>'Lateral'</th>
<th>'Vertical'</th>
</tr>
</thead>
<tbody>
<tr>
<td>VH</td>
<td>-5.2</td>
<td>4.8</td>
<td>8.0</td>
</tr>
<tr>
<td>VLT</td>
<td>-2.1</td>
<td>2.4</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Table 2(b): Stereotaxic co-ordinates for probe implantation sites.

Key: VH = Ventral hippocampus; VLT = Ventrolateral thalamus

' - positions relative to bregma. ' taken from surface of dura.
Probes were implanted unilaterally into the brain without the prior insertion of guide cannulae. The probe was mounted in a holder (in-house design), which was then attached to a micro-manipulator, which was used to lower the probe gradually the required depth into the brain. During this process, the probe was gently infused with aCSF to increase rigidity of the dialysis membrane. The probe was secured with methyl acrylic cement (Heathco, Bolton, Lancs.), which also covered the anchor screws, thus aiding the adherence of the cement to the skull surface. Cement was also built up around the shaft of the probe to add durability.

The cement was allowed to harden before the animal was removed from the frame, wrapped in a paper towel to prevent post-operative hypothermia, and placed individually onto sawdust bedding in a clear perspex box. Access to food and drinking water was ad libitum. Animals were allowed an overnight recovery period (18 ± 2 hrs). This extended period of recovery was allowed since acute implantation results in tissue damage, principally oedema (Edvinsson et al., 1971). Thus, acutely implanted microdialysis probes often recover excessive quantities of analytes which do not reflect true estimates of extracellular basal levels. In support of these findings, Westerink and De Vries (1989) have demonstrated that dialysate neurotransmitters recovered following acute probe implantation, are largely derived from damaged nerve terminals rather than steady-state release from neuronal stores.

2.2.4 Microdialysis

In all dialysis experiments, rats were housed individually in purpose built perspex boxes (30 x 30 x 30 cm), with a 2 cm diameter hole in the lid through which infusion and collection lines could be passed. All experiments were performed using freely moving animals which displayed no signs of distress following surgery. (Animals remained in the same cages as those they were put into after implantation so that re-acclimatisation would not be necessary).

The day following probe implantation, the inlet tubing of the dialysis probe was
connected to a 40 cm length of pp10 polyethylene tubing which had been flushed with, and thus contained, aCSF. This in turn was connected to a gas-tight 500 µl Hamilton microsyringe containing aCSF (with a small piece of peristaltic pump tubing), which was mounted on a 2-channel micro-infusion pump (Harvard Apparatus, syringe infusion pump 22). The dialysis probe was infused manually with aCSF for a short period of time to expel air bubbles, which if trapped inside the membrane would limit the area of diffusion, and secondly would reduce the volume of sample collected.

The outlet tubing of the probe was connected to a 25 cm length of FEP tubing (dead space 0.1 µl/cm; Carnegie Medicin, Sweden), which is relatively inert and thus prevents the adsorption of substances to the walls of the tubing. This was fed into a collecting vial. Both inflow and outflow lines were weighted with Blu-tack in an attempt to prevent the animals from knawing them.

If probes were functioning, the entire system was manually flushed with aCSF, for the purposes of washing and to check for a lack of resistance to the flow of aCSF, and further to expel any air bubbles. Before commencing microdialysis experiments, aCSF was infused via the probe at a rate of 0.5 µl/min. (to minimise interference with the normal physiology of the brain and to maximise recovery of neurochemicals) using the Harvard infusion pump, and in all experiments an initial 60 min. stabilisation fraction would be collected and discarded. This procedure served as a "wash-out" fraction to eliminate abnormally high levels of analytes, which would have accumulated in the vicinity of the dialysis membrane during the post-operative recovery period due to local tissue damage, before stable baseline levels are achieved. Consequently, dialysis samples collected thereafter would reflect an equilibration between the infusion medium (within the probe) and the extracellular space (surrounding the probe), which occurs in the absence of flow in the infusion medium (Benveniste and Hüttemeier, 1990). Following this, dialysis samples were collected manually at regular intervals (30 min.). It was necessary to compromise between sensitivity (i.e. recovery of neurotransmitters) and time resolution in selecting flow
rate, and consequently this relatively large time interval of sample collection was required for the analytical system employed, but which did not dilute the neurochemical content of the dialysates below the detection limit of the HPLC system. Samples were either analysed immediately by HPLC or refrigerated at -80°C, together with aliquots of the appropriate standards, to prevent breakdown of substances (see Fig. 2(b) for a schematic representation of the microdialysis technique). Methodological differences between separate studies employing microdialysis are described in detail in the appropriate results chapters.

2.2.5 Anatomical verification of probe placement

Microdialysis experiments were concluded by determining the localisation of the microdialysis probe in the brain.

Rats were killed by an overdose of pentobarbitone (Expiral 150 mg/kg i.p.), and their brains removed and frozen in isopentane at -40°C. Coronal brain sections (10 μm) were cut on a cryostat and mounted on gelatin-coated slides. Verification of probe placement was made by visualisation of the probe tract.

A typical probe placement is shown in Fig. 2(c), and in which the tract of the stainless steel shaft of the probe can be clearly seen entering the hippocampus.

Only data from animals with correctly located dialysis probe tracts were incorporated in the final analysis.
Fig. 2(b): A schematic representation of a microdialysis experiment and HPLC analysis.
Fig. 2(c): Photomicrograph illustrating a typical microdialysis probe placement.
The arrow shows the entry of the probe shaft into the hippocampus, with the membranous area (area for diffusion) extending 4 mm below this.
(Total magnification x 15).
2.3 High Performance Liquid Chromatography

Since microdialysis provides a highly filtered low volume, aqueous solution of polar analytes, dialysates can be directly and automatically accommodated by high performance liquid chromatography.

2.3.1 Reverse - Phase High Performance Liquid Chromatography

All dialysates obtained from in vitro recovery and in vivo experiments, were analysed for amino acid and monoamine content by HPLC with fluorimetric detection (HPLC-FD) of derivatised amino acids and electrochemical detection (HPLC-EC) respectively. Dialysis samples which could not be analysed during the course of experiments were frozen at -80 °C together with aliquots of the appropriate standards. (Monoamine samples were maximally stored up to 1 week, whilst amino acid samples were analysed within 1 month of collection).

2.3.2 Analysis of Amino acids by HPLC-FD

Separation of amino acid-containing samples was carried out by a gradient, reverse-phase HPLC system using the method of Lindroth & Mopper (1979).

2.3.2.1 Principle

Amino acids are not naturally fluorescent, therefore OPA (O-phthalaldialdehyde) is reacted with the primary amine group of amino acids in the presence of a thiol-reducing agent (2-mercaptoethanol), to form highly fluorescent molecules at alkaline pH. After column separation, the fluorescent derivatives may be detected using a fluorescent detector (see Fig. 2(d)).
2.3.2.2 Preparation of OPA-thiol Reagent

The OPA-thiol reagent was prepared at least 24 h before use as follows: 27 mg OPA (Fluka) was dissolved in 500 μl of absolute alcohol. 5 ml of 0.1 M sodium tetraborate (borax) were then added and the solution mixed. Finally, 50 μl mercaptoethanol was added in a fume cupboard and the solution mixed thoroughly, and since it is photo sensitive, stored in a darkened vial at 4 °C, until used.

2.3.2.3 Apparatus

The apparatus consisted of: Two Gilson solvent delivery pumps (model 303), a Dynamax C18 reverse phase column (5 μm ODS particles, 15 cm x 4.6 mm; Rainin Dynamax Instrument Corporation Inc., U.S.A.) with column heater (30 °C; Anachem), a 5 μm C18 guard column (Rainin Dynamax) (to protect the analytical column from strongly adsorbed compounds and fine particles), a Gilson refrigerated auto-injector (model 231)
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equipped with a Rheodyne injection valve and a Gilson data collection unit (model 621) which acted as an interface to a Dell personal computer (PC) system. The PC system was equipped with Gilson chromatography software with inbuilt gradient manager.

2.3.2.4 The Chromatographic System

2.3.2.4.1 Gradient system

A gradient separation was employed for the elution of amino acids, such that the mobile phase became progressively non-polar, and thus accelerated elution time. This allowed for the separation of acidic (e.g. ASP, GLU) through to relatively non-polar amino acid derivatives (e.g. GABA) from dialysate samples over a 40 min. run time. At the end of each run, a final increase in the methanol gradient washed off any remaining sample from the column, before returning to 100 % phosphate buffer (A), to allow re-equilibration for the next sample (see Fig. 2(f)).

2.3.2.4.2 Sample Analysis

Two mobile phase components, A (50 µM sodium dihydrogen orthophosphate, pH 5.5, 20 % methanol) and B (100 % methanol) were mixed prior to delivery to the column inlet. Before use, both mobile phases were filtered through 0.2 µm nitrocellulose membrane filter discs (Anachem), and were continually degassed by sparging with helium. The proportions of A and B in the mixture were determined by the relative outputs of the two mobile phase delivery modules, both of which were under the control of the software gradient manager. Samples for analysis (15 µl) were manually aliquoted into amber crimp top vials (Anachem) and placed in the movable rack of the autosampler (5 ± 2 °C), where each sample in turn was reacted with 15 µl of OPA-thiol reagent. Following a 2 min. delay, the mixture (30 µl) was injected onto the column via the Rheodyne syringe loading sample injector (15 µl loop). Co-incident with injection of the derivatised sample, the PC system was activated to begin the
gradient manager and data collection software.

2.3.2.4.3 Detection

The OPA-thiol amino acid conjugates were detected fluorimetrically (360 nm excitation filter; 455 nm emission filter).

Upon completion of the separation, the entire cycle was repeated automatically, until all samples had been analysed. Chromatograms and peak area data were generated as printouts by the software program.

2.3.2.5 Quantification of amino acids

Amino acid peaks were quantified by measuring peak area (software-generated). A low gain setting on the detector (to ensure larger peaks were accurately measured) was employed. The concentration of amino acids in dialysates was calculated from amino acid standards. Mixed amino acid standards (ASP, GLU, GLN, TAU and GABA; 2.5 mM) were stored as 1 ml aliquots at -80 °C, and diluted to 2.5 x 10^{-6} M on the required days, and run at regular intervals amongst dialysate samples. Standard curves run for each amino acid showed that the fluorescence response was linear over a wide range of concentrations. (see Fig. 2(e)).

2.3.2.6 Identification and Verification of peaks

Identification of peaks was achieved by comparison of retention times with authentic amino acid standards (see Fig. 2(f) for a typical amino acid standard).
Fig. 2(e): Typical standard curves for selected amino acids.
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Fig. 2(f): Typical mixed amino acid standard.

The dashed line represents the gradient used for elution of amino acids, together with the ordering and approximate retention times of selected amino acids.
2.3.3 Analysis of Monoamines using HPLC-ED

2.3.3.1 Principle

Monoamines are electroactive, but exhibit significant differences in their oxidation and reduction current/voltage (C/V) curves. These differences are used in the HPLC-EC separation technique for monoamines in dialysates (see Fig. 2(g)).

2.3.3.2 Apparatus

The HPLC system for the detection and quantification of dialysate monoamines and their metabolites consisted of the following components:

A Gilson solvent delivery module (model 303), a C\textsubscript{18} reverse phase column (3 \textmu m particle size ODS; 4.6 mm I.D. x 100 mm; Rainin Dynamax Instrument Co. Inc., U.S.A.) protected by a microsorb guard column (4.6 mm I.D. x 15 mm; C\textsubscript{18} 5 \textmu m; Rainin Dynamax Instrument Co. Inc., U.S.A.) and an ESA Coulochem model 5100A electrochemical detector with model 5011 porous graphite electrode analytical cell (Severn Analytical, Bedfordshire, U.K.). Both column and analytical cell were protected by an ESA model 5020 guard cell. Data were collected and processed using a PC system (Dell Corporation, U.S.A.) interfaced to the electrochemical detector (ECD) via a data collection unit (Drew Ltd., U.K.). Additionally, a BBC Goerz Metrawatt SE 120 chart recorder was coupled directly to the ECD output. This allowed a further 10-fold amplification of the ECD output, and was used, when necessary, for the quantification of dopamine and 5-HT. The chromatograms generated using the PC software (Drew Roseate software) were sufficient for quantification of DOPAC, HVA and 5-HIAA.
Fig. 2(g): Current/voltage curves for the oxidation and reduction of dopamine and 5-HT and their metabolites.

Current is expressed as a % of the maximal response.
2.3.3.3 The Chromatographic System

2.3.3.3.1 Sample Analysis

The separation technique was based on that of Hutson et al. (1989) with modifications to allow concurrent detection of dopamine and its metabolites. All separations were isocratic, and the prefiltered mobile phase consisted of: 90 mM sodium acetate, 35 mM citric acid, 0.34 mM EDTA and 0.06 mM sodium octylsulphonic acid (as ion-pairing reagent) with a final concentration of 12 % methanol, pH 4.2. Continually recycled, helium-degassed mobile phase was pumped at a flow rate of 1.0 ml/min.

2.3.3.3.2 Detection

A guard cell set at +500 mV was placed ahead of the column and analytical cell (this was necessary to reduce background interference by pre-oxidation of the mobile phase ahead of the injector). Electroactive species were detected by the analytical cell. The ECD settings were: Electrode 1: -175 mV (DOPAC is present in significantly higher concentrations than dopamine in ventral hippocampal dialysates, therefore at this reduction potential ~ 40 % DOPAC is reduced, thus the peak is not in danger of saturating, as compared to ~ 80 % reduction of dopamine, whilst 5-HT, 5-HIAA and HVA are reduced very little). The second electrode was set at +400 mV (an optimal sensitivity for the oxidation of 5-HT, 5-HIAA and HVA).
2.3.3.4 Quantification of Monoamines

The HPLC-ED system was regularly calibrated with standard solutions of monoamines and their metabolites, to ensure consistent identification of the correct peaks following analysis of dialysates. Stock mixed standard solutions (0.5 mM) were prepared in 0.1 M HCl and stored as 1 ml aliquots at -80 °C. Aliquots were then removed on the required days, and diluted in double-distilled de-ionised water to give a final concentration of 50 pmol/ml, and stored on ice ready for use. Dilutions of the standard mixture were injected manually into the HPLC-ED system via an injection valve (Rheodyne, U.S.A.) with a 10 μl loop (see Fig. 2(h) for monoamine standard curves). The loop was always overfilled (approximately 15 μl of standard mixture or dialysate) to ensure reproducibility. Peak areas and retention times were calculated using the PC chromatography software. Fig. 2(i) illustrates a typical separation of a monoamine standard mixture.

2.3.3.5 Identification and Verification of Peaks

Identification of peaks was achieved as with amino acids, by comparison of retention times with authentic monoamine standards (see Fig. 2(i)).
Typical standard curves for dopamine and 5-HT and their respective metabolites.
Fig. 2(i): Typical separation of a monoamine standard mixture.

KEY: (1) Dopamine   (5) HVA
     (2) DOPAC
     (3) 5-HT
     (4) 5-HIAA
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2.4 Thermospray Liquid Chromatography-mass Spectrometry

Due to the breadth of polarities that a drug and its metabolites cover, the most widely used method for drug analysis is reverse-phase high performance liquid chromatography utilising UV and radiochemical detectors. However, thermospray liquid chromatography-mass spectrometry (LC-TSP-MS) provides a more sensitive method for achieving this, and therefore was employed for determining the LTG concentrations achieved in sub-chronically (5 days) and chronically- (21 days) treated brain, plasma and microdialysate samples (see Chapters 6 and 4).

High performance liquid chromatography-mass spectrometry (HPLC-MS) analyses were kindly performed by Mr Roy Clare at The Wellcome Research Laboratories, Beckenham, Kent.

2.4.1 Apparatus

HPLC-MS:
A Hewlett Packard 1090 HPLC coupled to a PE Sciex API-III triple quadrupole mass spectrometer was used.
The following HPLC conditions were employed:
Column (Phenominex, U.K.) 30 x 4.6 mm i.d. Spherisorb SCX cation exchange
Solvent acetonitrile/25 mmole/L ammonium formate pH 4.0 (1:1)
Flow Rate 1 ml/min.
Column Temperature 40 °C

2.4.2 Standard Solutions

LTG:
Standard solutions in the range 0.01 - 10 g/ml, used for preparing spiked plasma and
brain standards, were prepared by dilution of a 1 mg/ml primary stock solution. All solutions were prepared in methanol.

Standard solutions for assaying microdialysate samples were prepared as follows: a 2.0 g/ml solution was prepared by dilution of a 1 mg/ml primary stock solution in aCSF/H₂O (3:2). Aliquots of this solution were then diluted with aCSF to give solutions with a range of concentrations between 0.05 - 1.0 g/ml.

Internal Standard:
A working standard solution of the internal standard (Wellcome compound 725C78 (see Fig. 2(j)), with a concentration of 0.1 g/ml in methanol was prepared by dilution of a 1 mg/ml primary standard solution.

Ammonium Formate buffer Solutions:
The pH of 25 mmole/L ammonium formate was adjusted to the required pH (3.5 or 4.0) by the addition of undiluted formic acid.

![Molecular structures of lamotrigine and the Internal standard (compound 725C78).](image)

**Fig. 2(j):** Molecular structures of lamotrigine and the Internal standard (compound 725C78).
2.4.3 Sample Preparation

Plasma and microdialysate samples were used without further treatment.

Brain samples were thawed and weighed, and distilled water was added at the rate of 2.5 ml/g of tissue. The samples were then homogenised, using a Silverson L4R homogeniser, to give a suspension containing approximately 286 mg/ml tissue. The suspensions were re-frozen for storage prior to analysis.

2.4.4 Extraction

Extraction of plasma samples:
Plasma samples (10 μl) were added to 1 ml of 10 mmol/L ammonium formate solution, to which 80 μl methanol and 10 μl internal standard solution had previously been added. After mixing and allowing to stand for approximately 10 min., the solutions were loaded slowly, under vacuum, onto 100 mg C₁₈ Bond-Elut solid phase extraction cartridges (Anachem), which had previously been activated by washing sequentially with 1 ml portions of acetonitrile, distilled water and 10 mmole/L ammonium formate (1 ml) and water (0.5 ml), the LTG was eluted with 750 ml acetonitrile/ 25 mmole/L ammonium formate, pH 3.5 (7:3 v/v).

Extraction of brain samples:
After thawing and mixing, 40 μl portions of brain homogenate (equivalent to approximately 11.4 mg of tissue) were added to 0.5 ml 10 mmole/L ammonium formate solution, to which 80 μl methanol and 10 μl internal standard solution had previously been added, in screw-cap Pyrex culture tubes. Ethyl acetate (2 mls) was then added and after capping, the tubes were vortex mixed for 1 h, and then spun at 5000 r.p.m. for 10 min. in a Sorrall RC-3D refrigerated centrifuge. The upper organic phase was taken off and blown to dryness under a stream of nitrogen at 60 °C, and the residue was reconstituted in 0.75 ml acetonitrile/ 25 mmole/L ammonium formate, pH 3.5 (7:3 v/v) for injection onto the HPLC.
2.4.5 Calibration Standards

Plasma:
A range of extracted standards were prepared in duplicate. These extractions were set up study samples, but using 10 μl of control rat plasma and replacing part of the 80 μl of methanol with suitable volumes of standard LTG solutions to give a final amount equivalent to plasma concentrations in the range 0.21 g/ml.

Brain:
As for the plasma assay, a range of extracted standards were prepared by using 80 μl of control rat brain homogenate (this had a tissue concentration one half that of the study brain homogenate), and replacing part of the methanol with standard LTG solutions, to give amounts of LTG in the range 5300 ng/ sample (equivalent to 0.4263 g/g of brain).

Microdialysates:
Solutions of LTG in aCSF, with concentrations in the range 0.052 g/ml, prepared as described earlier (see Section 2.4.2), were used without further treatment.

See Fig. 2(k) a-c for standard curves. The calibration data for the plasma standards gave a good fit to a weighted (x⁻¹) linear regression, but the calibration data for brain extracts and microdialysate samples had to be fitted to a quadratic model, indicating that it would have been better to have injected rather less sample.
Fig. 2(k): LTG calibration standard curves.
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2.4.6 HPLC-MS

50 μl of plasma and brain extracts, and 10 μl of dialysate samples were injected and the run time was 2.5 min. The mass spectrometer was fitted with a heated nebuliser (HN) interface operated with a nebuliser temperature of 500 °C, an interface temperature of 50 °C and an orifice voltage of 50 V. The instrument was used in the selection reaction monitoring (SRM) mode with argon collision gas and a collision energy of 20 eV.

LTG was monitored as the transition of its protonated molecule (m/z 256) to an ion of m/z 211, and the internal standard was monitored as the transition of its protonated molecule (m/z 218) to an ion of m/z 186. The transitions were monitored with a dwell time of 150 ms/transition.

2.5 Electroencephalograph (EEG) Recordings

2.5.1 Bipolar EEG recording electrode implantation

Bipolar EEG recording electrodes (MS 303/1; Semat Technical (U.K.) Ltd.) were implanted into the ventrolateral thalamus of GAERS under chloral hydrate anaesthesia, as previously described (see Section 2.2.3) for microdialysis probes. A dust cap was screwed onto the electrode surface, and animals allowed an overnight recovery as for dialysis experiments.

2.5.2 EEG Recording Apparatus

The following day, the dust cap was removed and a bipolar electrode cable (305-202/2; 50 cm length; Semat Technical (U.K.) Ltd.), which was surrounded by a stainless steel covering to protect against knawing, was connected to the electrode, under a 2 % halothane/1L oxygen anaesthetic mixture, and the animals allowed to recover.
Additionally, the sawdust bedding was replaced by tissue paper soaked in 0.9 % saline solution.

The stainless steel coil surrounding the recording cable, the animal itself, and the Faraday cage surrounding the complete preparation, were earthed to a single point to eliminate earth loops and movement artefacts (see Fig. 2(l)). Animals were allowed 60 min. to habituate to their surroundings before EEG recording was commenced, and were prevented from sleeping by gentle sensory stimulation (e.g. handling, random sounds, light etc.).

2.5.3 EEG Recording/ Analysis System

The signal was fed into an AC pre-amplifier (Neurolog NL-824) before being further amplified (1-30 Hz), and continuously displayed on an oscilloscope, with segments of interest being recorded onto chart paper. The amplified and filtered signal was also digitised (128 Hz sampling frequency) using a Dell microcomputer equipped with a DAS-801 data acquisition board (Keithley Instruments). A dedicated application programme (Programming language, Asyst version 4.0) allowed EEG data to be continuously acquired, transformed, displayed and stored for later analysis. Linear spectra (0-21 Hz) of consecutive EEG data sections (4 s) were compared using Fast Fourier Transformation, and continuously displayed (see Fig. 2(m)). Typical SWDs, as noted from the raw EEG signal on the oscilloscope, were easily recognisable by a peak in the 6-9 Hz window of the linear spectra, allowing this peak to be used for calculating the cumulative duration of SWDs within each recorded time interval (see Chapter 6).
Fig. 2(1): A schematic representation of an EEG recording set-up (see text for details).
Fig. 2(m): Typical EEG spike-and-wave discharges from the ventrolateral thalamus of GAERS.

SWDs are represented by peaks in the 6-9 Hz window (lower panel) and symbols above the base-line (upper panel) in the continuously displayed linear spectrum, which is calculated by means of a fast fourier transformation (see text for details). Data from two separate GAERS is represented.
2.5.4 Anatomical verification of EEG Recording Electrode placement

Verification of the correct positioning of EEG recording electrodes was confirmed as previously described for microdialysis probes (see Section 2.2.5).

2.6 Data and Statistical Analysis

All analysis was performed using IBM compatible software. Raw data was analysed with a spreadsheet package (Quattro Pro.; Borland) and Fig. P. (Biosoft, Cambridge) was used for graph production.

Statistical analysis was performed using Instat (Graphpad). Statistical significance was determined at the 5 % level. Details of statistical investigation are described in individual results chapters as appropriate.
CHAPTER 3

DIFFERENTIAL EFFECTS OF ACUTE LAMOTRIGINE ADMINISTRATION ON DEPOLARISATION-EVOKED NEUROTRANSMITTER EFFLUX FROM THE RAT VENTRAL HIPPOCAMPUS IN VIVO: FUNCTIONAL IMPLICATIONS
3.1 Introduction

The novel antiepileptic drug lamotrigine (LTG) has a broad spectrum of efficacy in both animal models of seizures (e.g. maximal electroshock, pentylenetetrazol, kindling) (Upton, 1994), and in human epilepsies (e.g. partial, secondarily generalised tonic-clonic, Lennox-Gastaut syndrome, juvenile myoclonic epilepsy) (Messenheimer, 1994; Yuen, 1994; Burstein, 1995). Additionally, it has been shown to exert neuroprotective effects in experimental models of focal and global ischaemic injury (Smith and Meldrum, 1995; Wiard et al., 1995), as well as to possess analgesic (Klamt et al., 1994) and anxiolytic (Critchley, 1994) properties.

However, despite extensive studies, the mechanism of action of LTG has yet to be fully elucidated. Several lines of investigation now strongly suggest that its primary site of action is at voltage-sensitive Na+ channels, and that this mechanism is fundamental for stabilising excitable neuronal membranes, thereby reducing pathologically-altered neurotransmission (reviewed by Meldrum and Leach, 1994; Leach et al., 1995). Early in vitro studies, for example, indicated that LTG inhibited veratrine-evoked, but not basal- or high K+-evoked, amino acid release, in particular glutamate, and less potently aspartate and GABA (Leach et al., 1986).

In support of these neurochemical findings, a number of other studies have reported LTG to be of benefit in pathological conditions in which the involvement of glutamate has been implicated. For example, McGeer and Zhu (1990) have reported that LTG provides protection against kainate neurotoxicity in the striatum, which is dependent upon the release of neuronal glutamate, whilst being ineffective against ibotenic acid-induced neurotoxicity, the mechanism of which is independent of glutamate release. Furthermore, LTG is capable of inhibiting the ischaemic-induced release of glutamate (Graham et al., 1993), as well as being of therapeutic benefit in several Parkinsonian patients (Zipp et al., 1993).

1antiparkinsonian drugs, such as memantine, may owe their action to a blocking of glutamatergic transmission (Kornhuber et al., 1989, 1991).
In addition, ligand binding studies on brain membranes and synaptosomes have also demonstrated a LTG interaction with Na⁺ channels (Leach et al., 1995). These findings have been confirmed and extended by electrophysiological investigations (Cheung et al., 1992; Lees and Leach, 1993), which have shown LTG to act as a use- and voltage-dependent inhibitor of type IIa Na⁺ channels (Xie et al., 1994, 1995). The effects of LTG on Ca²⁺ currents have however been less consistently reported. LTG has been shown to inhibit a "presumptive" Ca²⁺ current in rat cortical cultures (Lees and Leach, 1993), whilst having no effect on either L- or T-type Ca²⁺ currents in whole cell clamped pituitary cells (Lang et al., 1993) at concentrations effective at blocking Na⁺ conductances. However, more recent evidence, has demonstrated an inhibition of N- and P-type Ca²⁺ currents in cortical neurones, also at therapeutically effective doses (Stefani et al., 1996). These findings therefore suggest that modulation of both Na⁺ and Ca²⁺ currents may be relevant for the mechanism of action of LTG.

Biochemically, it is widely considered that epileptic seizures may arise from excessive, or increased glutamatergic neuronal tone (Meldrum and Garthwaite, 1990; Meldrum, 1994), or from a reduction in GABA-mediated inhibitory mechanisms, prolonging neuronal excitability as well as leading to the generation of epileptiform activity (Kreigstein, 1988; Rogawski and Porter, 1990; Carlsson et al., 1992; Meldrum, 1994). In addition, the role of central monoaminergic systems (e.g. 5-HT, dopamine) in the pathophysiology of epilepsy has been extensively studied (see Section 1.3.3.2.2). Reductions in 5-HT and dopamine levels have been demonstrated in both normal and kindled animals (Anlezark and Meldrum, 1975; Corcoran and Mason, 1980; Cavelheiro et al., 1981), as well as in animals genetically prone to convulsive or non-convulsive epilepsies (Meldrum et al., 1975; Laird et al., 1983; Jobe et al., 1984).

Thus to clarify further the neurochemical properties of LTG, its effects on basal- and depolarisation-induced extracellular amino acid and monoamine efflux from the rat ventral hippocampus, have been examined using in vivo microdialysis. Two depolarising agents differing in their release mechanisms - elevated K⁺ (producing release of neurotransmitters from synaptic terminals in a largely Ca²⁺-dependent
manner (Nicholls, 1993)) and veratridine (release occurring by enhanced Na⁺ entry) - were employed. The hippocampus was chosen for these studies as it is particularly prone to the generation of epileptiform activity (McNamara, 1994), as well as being a major centre for the genesis and maintenance of kindled seizures (Minamoto et al., 1992). In addition, this neuronal region is closely associated with inhibitory and/or excitatory neurotransmitter abnormalities during the occurrence of epileptiform activity (Dichter, 1989; Meldrum, 1990, 1995; Ronne-Engstrom et al., 1992; Löscher, 1993; Rowley et al., 1995b).
Chapter 3: Acute effects of LTG on neurotransmitter efflux

3.2 Methods

Male Wistar rats, implanted with microdialysis probes in the ventral hippocampus, and having recovered for 18-20 h post-operatively, were used in all microdialysis experiments.

3.2.1 Experimental protocols

3.2.1.1 Effects of lamotrigine on basal amino acid and monoamine efflux

A total of 12 x 30 min. consecutive, dialysate samples were collected. Lamotrigine isethionate, or its vehicle (distilled water) were injected intraperitoneally (i.p.) after the collection of 4 x 30 min. basal samples, and then 8 x 30 min. post-injection samples were collected.

3.2.1.2 Effects of lamotrigine on depolarisation- (high K+/veratridine) evoked amino acid and monoamine efflux

A total of 15 x 30 min. samples were collected. The following infusion regimen was employed for this purpose:

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>aCSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4</td>
<td>normal*</td>
</tr>
<tr>
<td>5</td>
<td>100 mM K+/ 50 μM veratridine (S1)</td>
</tr>
<tr>
<td>6-7</td>
<td>normal</td>
</tr>
<tr>
<td>→LTG/vehicle administration i.p.</td>
<td></td>
</tr>
<tr>
<td>8-10</td>
<td>normal</td>
</tr>
<tr>
<td>11</td>
<td>100 mM K+/ 50 μM veratridine (S2)</td>
</tr>
<tr>
<td>12-15</td>
<td>normal</td>
</tr>
</tbody>
</table>

Table 3(a): aCSF infusion protocol used to investigate the effects of LTG on depolarisation-evoked amino acid and monoamine efflux.

*aCSF for monoamine analysis contained 1μM citalopram.
Miller et al. (1986) have previously shown LTG to be maximally effective 1 h post-injection at preventing MES-induced seizures, therefore LTG was administered 90 min. prior to the second period of veratridine/ K\(^+\) infusion in the present study.

Alternate infusion solutions were placed in a second syringe in the microdialysis pump, and manually switched over at the appropriate times, by disconnecting the tubing supplying aCSF from the inlet of the dialysis probe, and rapidly connecting one supplying aCSF containing the depolarising stimulant. Following the cessation of stimulation, there was a return to the original dialysis solution. Depolarising solutions were prepared either by simply adding the substance to the aCSF (veratridine), or by replacing one ion in the aCSF with another, as in experiments with high K\(^+\) stimulation, in which the KCl concentration was raised to 100 mM, whilst the NaCl concentration was reduced to 27.5 mM in order to maintain isotonicity. The concentrations of veratridine and high K\(^+\) which were used, were sufficient to elicit a measurable, but not excessive, release of transmitters in vivo (Badoer et al., 1989; Paulsen and Fonnum, 1989; Fairbrother et al., 1990; Matos et al., 1990; Sorkin et al., 1991; Bourdelais and Kalivas, 1992; Hondo et al., 1995).

3.2.2 Biochemical Analysis

Dialysate samples were assayed, by HPLC-EC for monoamines, and by HPLC-FD for amino acids, as previously described in Section 2.3.

3.2.3 Statistical Analysis

All data were calculated as a percentage increase or decrease of basal efflux, and analysed for statistical differences between drug- and vehicle-treatments at each time point, using a Mann-Whitney U test. In experiments investigating stimulated transmitter efflux, the changes produced during the two periods of stimulation (S1 and S2), were calculated as the difference between the sample immediately prior to
stimulation (i.e. samples 4 and 10), and the levels measured during the stimulation period itself. The changes produced by the second (S2) and the first (S1) stimulation periods were expressed as a ratio (S2/S1) and compared between treatment groups (Mann-Whitney U test). All values are means ± s.e. mean, with p<0.05 considered to be statistically significant.
Chapter 3: Acute effects of LTG on neurotransmitter efflux

3.3 Results

3.3.1 Effects of lamotrigine on extracellular amino acid efflux

Basal amino acid concentrations in ventral hippocampal dialysates were calculated from the mean values in the first four 30 min. samples.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Conc. (pmols/15μl dialysate/30 min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>4.30 ± 0.80</td>
</tr>
<tr>
<td>Glutamate</td>
<td>36.20 ± 4.80</td>
</tr>
<tr>
<td>Glutamine</td>
<td>49.20 ± 6.30</td>
</tr>
<tr>
<td>Taurine</td>
<td>106.0 ± 3.40</td>
</tr>
<tr>
<td>GABA</td>
<td>2.90 ± 1.0</td>
</tr>
</tbody>
</table>

Table 3(b): Basal extracellular amino acid concentrations in the rat ventral hippocampus. Values are means ± s.e. mean (n = 56).

The basal efflux of the transmitter amino acids, aspartate and GABA were several orders of magnitude lower than that of the non-transmitter amino acids taurine and, less so, glutamine.

3.3.1.1 Effects of lamotrigine on basal extracellular amino acid efflux

13.4 mg/kg i.p. LTG base (n=6) had no effect on amino acid efflux. However, at the higher dose of 26.8 mg/kg i.p. LTG base (n=6), there was a small, statistically non-significant decrease in basal aspartate concentration, but a significant decrease (maximal 40 %) in dialysate glutamate concentration (Fig. 3(a)). The LTG-induced reduction in glutamate concentration began 30 min. post-administration, and persisted for 210 min., before gradually returning to basal levels. Also at this dose, all rats displayed an abnormally flattened body posture with an abnormal spread of the legs, and were unresponsive to their surroundings for up to 60 min. post-administration.
Chapter 3: Acute effects of LTG on neurotransmitter efflux

A: Percentage of basal ASP efflux over time (0-360 min).
- Vehicle
- 13.4 mg/kg LTG
- 26.8 mg/kg LTG

B: Percentage of basal GLU efflux over time (0-360 min).
- Vehicle
- 13.4 mg/kg LTG
- 26.8 mg/kg LTG

C: Percentage of basal GLN efflux over time (0-360 min).
- Vehicle
- 13.4 mg/kg LTG
- 26.8 mg/kg LTG
Fig. 3(a): Effects of acute LTG administration on basal extracellular amino acid efflux (A-E) from the rat ventral hippocampus.

For each time-point, mean ± s.e.mean (n=6/group) is expressed as a % of the respective basal amino acid concentration. The arrows denote the time-points at which LTG/vehicle (distilled water) was administered i.p.; *p<0.05, represents a significant difference from vehicle effects (Mann-Whitney U test).
3.3.1.2 Effects of lamotrigine on depolarisation-evoked amino acid efflux

3.3.1.2.1 Effects of lamotrigine on veratridine-evoked amino acid efflux

A 30 min. infusion of veratridine (50 μM; n=5-6/group), via the microdialysis probe was accompanied by marked behavioural activation, characterised by increased grooming, exploration, and in some animals periods of stillness mid-motion and "wet-dog" shakes for up to 30 min. post-stimulation. Additionally, veratridine elicited an increase in the efflux of aspartate (to 349 ± 54 %), glutamate (to 206 ± 26 %), taurine (to 196 ± 21 %) and GABA (to 1824 ± 480 %) above basal levels. In contrast, glutamine levels decreased below the baseline (to 61 ± 5 %). The maximal effects of stimulation were observed in sample 6, before gradually returning to baseline levels in the subsequent two samples. Generally, after depolarisation, the extracellular levels of the transmitter amino acids, aspartate, glutamate and GABA, returned to basal levels faster than taurine and glutamine. *(see Fig. 3(c)).

The second stimulation with veratridine (S2) also elevated aspartate (to 205 ± 36 %), GABA (to 1147 ± 227 %) and taurine (to 154 ± 16 %) efflux above basal levels. The magnitude of these changes was less than that achieved during S1, despite the basal fractions (samples 4 and 10) immediately prior to veratridine stimuli not being significantly different. In contrast, veratridine evoked a sustained increase in glutamate efflux, which reached a maximum (to 324 ± 63 %) 30 min. post-infusion, before decreasing slightly, and then persisting at a significantly elevated level for the remainder of sampling. Glutamine levels in contrast, were reduced to the same extent as achieved during S1 (to 54 ± 7 % of basal levels). The resulting S2/S1 ratios in the control group were: aspartate (0.59 ± 0.13), glutamate (1.57 ± 0.38), glutamine (0.89 ± 0.16), taurine (0.79 ± 0.22) and GABA (0.62 ± 0.18) *(N.B. the failure of S2-evoked glutamate efflux to return to baseline levels contributed to the significantly greater than 1.0 S2/S1 ratio (see Fig. 3(h)).
Pretreatment with 6.70 mg/kg LTG did not significantly alter S2 veratridine-evoked amino acid efflux when compared to the control group. However, following administration of 13.4 mg/kg LTG, rats were less behaviourally active than those of the control group in response to veratridine stimulation (S2).

<table>
<thead>
<tr>
<th>Behaviours</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
</tr>
<tr>
<td>Grooms</td>
<td>10.0 ± 3.0</td>
</tr>
<tr>
<td>Cage crossings</td>
<td>20.0 ± 4.30</td>
</tr>
<tr>
<td>&quot;wet-dog&quot; shakes</td>
<td>30.0 ± 5.70</td>
</tr>
<tr>
<td>Feed bouts</td>
<td>4.0 ± 3.20</td>
</tr>
</tbody>
</table>

Table 3(c): Veratridine (50 μM)-evoked behavioural responses.
Effects of acute LTG- (13.4 mg/kg i.p.) or vehicle- (distilled water) administration on veratridine-evoked behavioural responses following infusion into the rat ventral hippocampus. Data are means ± s.e.mean (n=6-8/group); *p<0.05 represents a significant difference from vehicle effects (Student’s t-test).

Concomitant with these observations, 13.4 mg/kg LTG, significantly reduced the maximal veratridine-stimulated elevations of aspartate (by 54 ± 16.56 %) and GABA (by 52 ± 18.9 %), and more modestly taurine efflux (by 46 ± 11.20%), compared to S1 evoked release (Fig. 3(b) a,d,e). However, the effect of LTG on veratridine-stimulated glutamate efflux was most marked at this dose, reducing the efflux to apparent basal concentrations (73 ± 19.4 %), whilst in contrast being ineffective at 6.70 mg/kg LTG. The significant reductions in stimulated aspartate and glutamate levels were long-lasting, persisting for the duration of sampling. The resulting S2/S1 ratios were: aspartate (0.46 ± 0.16), glutamate (0.42 ± 0.11), glutamine (0.77 ± 0.20), taurine (0.54 ± 0.13) and GABA (0.34 ± 0.09). These results are summarised in Fig. 3(h). The difference in the S2/S1 ratios between LTG- and vehicle-treatment groups were significant for aspartate, glutamate, taurine and GABA.
Chapter 3: Acute effects of LTG on neurotransmitter efflux

A: 450 - vehicle
- 6.7 mg/kg LTG
- 13.4 mg/kg LTG

B: 450
- 6.7 mg/kg LTG
- 13.4 mg/kg LTG

C: 120
- 13.4 mg/kg LTG
Fig. 3(b): Effects of acute LTG-administration on veratridine-evoked (50 µM) amino acid efflux (A-E) from the rat ventral hippocampus.

The bars marked "V" represent the time periods in which veratridine was infused through the microdialysis probe, whilst the arrows depict the time-points at which LTG/vehicle was administered i.p.; *p<0.05, **p<0.01, represent significant differences from vehicle effects (Mann-Whitney U test).
Fig. 3(c): Representative amino acid chromatograms from the rat ventral hippocampus.  
(A) Effects of no treatment and (B) effects of 50 µM veratridine on amino acid efflux.
Chapter 3: Acute effects of LTG on neurotransmitter efflux

Fig. 3(d): Representative amino acid chromatogram from the rat ventral hippocampus.

(C) effects of acute LTG administration (13.4 mg/kg i.p.) on veratridine-evoked (50 μM) amino acid efflux (see text for details).
3.3.1.2.2 Effects of lamotrigine on K⁺-evoked amino acid efflux

K⁺ infusion (100 mM) through the probe into the ventral hippocampus, led to behavioural effects comparable to those exhibited post-veratridine stimulation. Concomitantly, K⁺ elicited marked increases in aspartate (to 266 ± 44 %), glutamate (to 198 ± 33 %), taurine (to 470 ± 140%) and GABA (to 911 ± 416 %), and a decrease in glutamine (to 69 ± 17 %) efflux of basal levels (n = 6-9) in the control group. The maximal effects were observed 30 min. post K⁺-stimulation, and levels returned to basal concentrations in the subsequent 60 min. The magnitude of release proceeding the second period of K⁺-stimulation (S2) was comparable to the first (S1) (Fig. 3(e)). Dialysate levels of aspartate (to 277 ± 34 %), glutamate (to 225 ± 66 %), taurine (to 357 ± 89 %) and GABA (to 804 ± 213 %) were once again markedly increased above basal levels in the 30 min. period subsequent to S2, whilst glutamine levels were significantly reduced (to 64 ± 14 %). The resulting S2/S1 ratios in the control group were: aspartate (1.04 ± 0.14), glutamate (1.14 ± 0.23), glutamine (0.93 ± 0.11), taurine (0.76 ± 1.08) and GABA (0.88 ± 0.08) (see Fig. 3(h)).

LTG administration (13.4 mg/kg; n=7/group) did not significantly alter the S2 K⁺-stimulated efflux of the measured amino acids. These results are summarised in Fig. 3(e).
Chapter 3: Acute effects of LTG on neurotransmitter efflux

A: 450

- vehicle
- 13.4 mg/kg LTG

% of basal ASP efflux

Time (min.)

K+ K+

B: 350

% of basal GLU efflux

Time (min.)

K+ K+

C: 140

% of basal GLN efflux

Time (min.)
Fig. 3(e): Effects of acute LTG administration on elevated K⁺-evoked (100 mM) amino acid (A-E) efflux from the rat ventral hippocampus.

The bars marked "K⁺" represent the time-periods in which K⁺ was infused through the microdialysis probe, whilst the arrows depict the time-points at which LTG/vehicle was administered i.p.
Fig. 3(f): Representative amino acid chromatograms from the rat ventral hippocampus. (A) effects of no treatment and (B) effects of 100 mM K+ on amino acid efflux.
Chapter 3: Acute effects of LTG on neurotransmitter efflux

Fig. 3(g): Representative amino acid chromatogram from the rat ventral hippocampus. (C) effects of acute LTG-administration (13.4 mg/kg i.p.) on K⁺-evoked (100 mM) amino acid efflux (see text for details).
Fig. 3(h): Selective inhibition of (A) veratridine- but not (B) K"-evoked amino acid efflux by LTG from the rat ventral hippocampus.

S2/S1 ratios (mean ± s.e.mean) were calculated from the depolarisation-evoked responses in the absence (S1) and in the presence (S2) of LTG. The asterisks denote ratios that are statistically different from those of the corresponding control values; *p<0.05, **p<0.01 (Mann-Whitney U test).
3.3.2 Effects of lamotrigine on extracellular monoamine efflux

Basal monoamine concentrations in ventral hippocampal dialysates were also calculated from the mean values in the first four 30 min. samples.

<table>
<thead>
<tr>
<th>Monoamine</th>
<th>Conc. (fmols/10 μl dialysate/30 min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td>54.70 ± 9.40</td>
</tr>
<tr>
<td>DOPAC</td>
<td>801.0 ± 143.0</td>
</tr>
<tr>
<td>HVA</td>
<td>1643 ± 463.0</td>
</tr>
<tr>
<td>5-HT</td>
<td>103.0 ± 13.20</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>9830.0 ± 883.0</td>
</tr>
</tbody>
</table>

Table 3(d): Basal extracellular monoamine (and their metabolites) concentrations in the rat ventral hippocampus.
Values are means ± s.e.mean (n=52).

The basal efflux of the neurotransmitters dopamine and 5-HT were several orders of magnitude lower than that of their respective metabolites.

3.3.2.1 Effects of lamotrigine on basal monoamine efflux

Administration of 13.4 mg/kg LTG (n=6) produced a dose-related, statistically non-significant decrease in basal extracellular levels of dopamine and its metabolites DOPAC and HVA. These drug-induced inhibitory effects began 30-60 min. post-administration (Fig. 3(i)), and at 26.8 mg/kg LTG were sustained for the duration of sampling. Despite an obvious downward trend, statistical significance at the higher dose was attained at only some time-points; maximal reductions were: dopamine (to 68 ± 13 %), DOPAC (to 60 ± 9 %) and HVA (to 74 ± 4 %) of baseline levels. In comparison, basal 5-HT and 5-HIAA levels were not significantly altered at 13.4 mg/kg LTG. At 26.8 mg/kg LTG, basal 5-HT levels were reduced within 30 min. post-administration (maximally to 53 ± 6 % of basal), and persisted for 210 min. In contrast, 5-HIAA levels remained unchanged.
Chapter 3: Acute effects of LTG on neurotransmitter efflux

![Graph A: % of basal dopamine efflux](image)

- **A:** 140
- % of basal dopamine efflux
- Time (min.): 0 60 120 180 240 300 360
- Symbols:
  - •: vehicle
  - ■: 13.4 mg/kg LTG
  - ◇: 26.8 mg/kg LTG

![Graph B: % of basal DOPAC efflux](image)

- **B:** 140
- % of basal DOPAC efflux
- Time (min.): 0 60 120 180 240 300 360
- Symbols:
  - •: vehicle
  - ■: 13.4 mg/kg LTG
  - ◇: 26.8 mg/kg LTG

![Graph C: % of basal HVA efflux](image)

- **C:** 140
- % of basal HVA efflux
- Time (min.): 0 60 120 180 240 300 360
- Symbols:
  - •: vehicle
  - ■: 13.4 mg/kg LTG
  - ◇: 26.8 mg/kg LTG
Chapter 3: Acute effects of LTG on neurotransmitter efflux

Fig. 3(i): Effects of acute LTG-administration on basal dopamine and 5-HT (and their metabolites) (A-E) efflux from the rat ventral hippocampus. The arrows denote the time-points at which LTG/vehicle was administered i.p.; *p<0.05, represents significant differences from vehicle effects (Mann-Whitney U test).
3.3.2.2 Effects of LTG on depolarisation-evoked monoamine efflux

3.2.2.2.1 Effects of lamotrigine on veratridine-evoked extracellular monoamine efflux

Infusion of veratridine (n=6/group) was associated with behavioural activation, elevations in dopamine (to 189 ± 12 %) and 5-HT (284 ± 32 %), with reductions in the levels of their respective metabolites, DOPAC (to 73 ± 8 %) and 5-HIAA (to 62 ± 5 %), but an increase in HVA (to maximally 210 ± 32 %) of basal levels. The maximal effects on stimulation-evoked dopamine and 5HT efflux were observed 30 min. post-infusion, and took up to 90 min. to return to baseline. The second stimulation with veratridine also elevated dopamine (to 141 ± 17 %) and 5-HT (to 168 ± 32 %) above basal levels. However, these changes were of a smaller magnitude than those achieved during S1. LTG administration (13.4 and 26.8 mg/kg) had no significant effect on veratridine-evoked (S2) dopamine and DOPAC, or 5-HT and 5-HIAA efflux compared to the vehicle-treated group. HVA efflux was however significantly reduced (maximally to 49 ± 10 %).

3.3.2.2.2 Effects of lamotrigine on K⁺-evoked extracellular monoamine efflux

Infusion of K⁺ (n=5/group), was also associated with behavioural activation, elevations in the release of dopamine (to 220 ± 43 %) and 5-HT (to 398 ± 91 %), and a decline in metabolite levels, DOPAC (to 74 ± 11 %), HVA (to 73 ± 15 %) and 5-HIAA (62 ± 2 %). The maximum elevations in dopamine and 5-HT efflux occurred up to 30 min. post-infusion and generally returned to basal levels in the subsequent sample. Similarly, the S2-evoked efflux of dopamine (to 190 ± 35 %) and 5-HT (to 274 ± 49 %) was reduced compared to that achieved during the S1 K⁺ stimulation. As with veratridine, K⁺-evoked monoamine efflux was not inhibited by LTG (13.4 and 26.8 mg/kg) (see Fig. 3(k)).
Chapter 3: Acute effects of LTG on neurotransmitter efflux

A: 300 - 150
  - vehicle
  - 13.4 mg/kg LTG
  - 26.8 mg/kg LTG

B: 140 - 100
  - 13.4 mg/kg LTG
  - 26.8 mg/kg LTG

C: 250 - 150
  - 13.4 mg/kg LTG
  - 26.8 mg/kg LTG

% of basal efflux

Time (min.)
Chapter 3: Acute effects of LTG on neurotransmitter efflux

Fig. 3(j): Effects of LTG on veratridine-evoked (50 μM) monoamine efflux (A-E) from the rat ventral hippocampus.

The bars marked "V" represent the time periods during which veratridine was infused through the microdialysis probes, whilst the arrows depict the time-points at which LTG/vehicle was administered; *p<0.05, **p<0.01, represent significant differences from vehicle effects (Mann-Whitney U test).
Chapter 3: Acute effects of LTG on neurotransmitter efflux

A: 350

% of basal dopamine efflux

• vehicle
■ 13.4 mg/kg LTG
△ 26.8 mg/kg LTG

Time (min.)

B: 140

% of basal DOPAC efflux

Time (min.)

C: 140

% of basal HVA efflux

Time (min.)
Fig. 3(k): Effects of LTG on K⁺-evoked (100 mM) monoamine efflux (A-E) from the rat ventral hippocampus. The bars marked "K⁺" represent the time periods in which K⁺ was infused through the microdialysis probes, whilst the arrows depict the time-points at which LTG/vehicle was administered i.p.
Chapter 3: Acute effects of LTG on neurotransmitter efflux

Fig. 3(1): Effects of LTG on (A) veratridine- and (B) K⁺-evoked monoamine efflux from the rat ventral hippocampus.

S2/S1 ratios (mean ± s.e.mean) were calculated from the depolarisation-evoked responses in the absence (S1) and in the presence (S2) of LTG. Asterisks denote ratios that are statistically different from those of the corresponding control values; *p<0.05 (Mann-Whitney U test).
Chapter 3: Acute effects of LTG on neurotransmitter efflux

3.4 Discussion

There is compelling evidence to suggest that epileptic seizures may be associated with neurochemical imbalances, resulting in long-term neuronal excitability and the generation of epileptiform activity (see Section 1.3.3.2). Previous studies on the novel antiepileptic drug LTG have led to the proposal that by inhibiting voltage-sensitive Na⁺ channel activity, LTG may be capable of stabilising excitable epileptic membranes, consequently reducing pathologically-altered neurotransmission (reviewed by Leach et al., 1995). The in vivo findings of the present study have demonstrated acute LTG administration to exert differential neurochemical effects upon basal- and depolarisation-evoked amino acid and monoamine efflux from the rat ventral hippocampus. These effects of LTG are discussed with relevance to possible anticonvulsant properties.

3.4.1 Effects of lamotrigine on basal extracellular amino acid and monoamine efflux

Besides a significant reduction in extracellular glutamate levels (40 %) at 26.8 mg/kg LTG, which is approximately 8-times the ED₅₀ dose required (3.60 mg/kg i.p.) in the rat MES test (Miller et al., 1986), LTG did not alter basal amino acid efflux. These findings would imply that at experimental anticonvulsant doses (Miller et al., 1986), basal amino acid efflux is relatively LTG-insensitive.

Neurotransmitter release has classically been accepted as being of a Ca²⁺-dependent, vesicular nature (Cohen and Van der Kloot, 1982; Llinas, 1982). However, dialysis studies (Westerink et al., 1987; Bourdelais and Kalivas, 1992; Timmerman et al., 1992) have demonstrated a significant portion of amino acid efflux to be tetrodotoxin (TTX)-insensitive and Ca²⁺-independent, suggesting that release consists of both neuronal as well as non-neuronal (possibly glial), and/or metabolically-derived components. Furthermore, more recently, Nicholls (1993) has demonstrated that a large
fraction of neuronal amino acids is located cytoplasmically, with release being Na⁺- (Szatkowski et al., 1990; Attwell et al., 1993) and not Ca²⁺-dependent (Belhage et al., 1993; Nicholls, 1993; Turner and Dunlap, 1995). Under certain conditions (both physiological and pathological), reversal of Na⁺-dependent amino acid uptake carriers, may be responsible for pumping transmitters out of cells in a Ca²⁺-independent, non-vesicular manner (Nicholls and Attwell, 1990; Attwell et al., 1993). However, at least 50% of the basal outflow of GABA for example, is considered to be of vesicular origin (Herbison, 1990; Bourdelais and Kalivas, 1992).

Since dialysis samples are representative of overall neurotransmitter efflux, and not just the neuronal release process, the observed total lack of an effect on basal extracellular amino acids (at anticonvulsant, side-effect free doses in rodents), would indicate that LTG does not alter either (a) probe recovery, (b) Ca²⁺-dependent vesicular release (exocytosis), (c) cellular re-uptake (with possible Ca²⁺-independent efflux due to reversed uptake) or (d) neurotransmitter metabolism of amino acids, in any significant way, so as to change net efflux. In contrast, monoamines are mainly stored in synaptic vesicles (Nicholls, 1989), and are released by Ca²⁺-dependent exocytosis (Westerink et al., 1987; Auerbach et al., 1989; Sharp et al., 1989, 1990; Matos et al., 1990; Nicholls, 1993). Since LTG (13.4 mg/kg) also did not significantly alter the basal (presumably neuronally-derived) efflux of the monoamines or of their metabolites, would further demonstrate a lack of effect upon basal Ca²⁺-dependent release processes, re-uptake or metabolism at experimental therapeutic doses. However, at the higher dose of 26.8 mg/kg LTG (at which behavioural side-effects were apparent), significant reductions in basal DOPAC, HVA and 5-HT did occur.

Generally, the lack of effect upon basal neurotransmitter efflux would suggest that normal physiological synaptic neurotransmission is not impeded by LTG at doses which protect against seizures in rodents.
3.4.2 Effects of lamotrigine on depolarisation-evoked amino acid and monoamine efflux

LTG had no significant effect on either K⁺-evoked amino acid or monoamine efflux, but potently inhibited the veratridine-evoked efflux of the amino acids but not the monoamines. These observations agree with an action at voltage-dependent Na⁺-channels for LTG (Nicholls, 1989), and would further suggest that inhibition appears to be modulated by channel activity (c.f. effects on basal efflux).

Raising extracellular [K⁺] produces a reversal of the K⁺ electrochemical gradient, and consequently Na⁺-independent membrane depolarisation. This process stimulates neurotransmitter release by activation of voltage-dependent Ca²⁺ channels, thereby inducing Ca²⁺-dependent exocytosis. In addition, Ca²⁺-independent release of amino acids can occur by reversal of amino acid uptake carriers, which normally countertransport K⁺ out of both neurones and glia (Nicholls, 1989; Paulsen and Fonnum, 1989; Patterson et al., 1995).

In comparison, veratridine increases membrane Na⁺ permeability by preventing the inactivation of neuronal Na⁺ channels, thereby enhancing Na⁺ uptake and producing a long-lasting depolarisation (Levi et al., 1980; Minchin, 1980). This is accompanied by a concomitant influx of Ca²⁺ (attributed to both voltage-sensitive Ca²⁺ channels and to reversal of the Na⁺-Ca²⁺ exchanger), and efflux of K⁺. Additionally, the Na⁺-concentration gradient is dissipated (which does not occur with elevated K⁺), resulting in a dramatic reversal of Na⁺-coupled transport processes, such as Na⁺: amino acid co-transport (Kalen et al., 1988; Fairbrother, 1990; Nicholls, 1993). During veratridine-induced depolarisation, Ca²⁺ influx elicits classical exocytotic release of both monoamine and amino acid neurotransmitters. Additionally, since veratridine reverses the Na⁺ electrochemical gradient by increasing intracellular [Na⁺] (Nicholls, 1989, 1993; Heron et al., 1995), amino acids can be released by reversal of Na⁺-dependent uptake carriers. This route of neurotransmitter liberation is consequently wholly Na⁺-dependent and Ca²⁺-independent, and furthermore, is the major contributor to amino
Chapter 3: Acute effects of LTG on neurotransmitter efflux

acid efflux (Jacobson and Hamberger, 1984; Nicholls, 1989). In contrast, Ca\(^{2+}\)-independent release has little role in monoamine efflux in view of the limited amount of "free" amines in the cytoplasm (Nicholls, 1989). The contribution of glia to veratridine-evoked neurotransmitter release remains unclear, although the existence of non-conducting Na\(^+\) channels, which can be activated by veratridine and blocked by TTX, has been shown (Reiser and Hamprecht, 1983; Yarowsky and Krueger, 1989). Whilst early studies suggested that veratridine did not promote transmitter release from glial cells (Minchin, 1975; Neal and Bowery, 1979), more recent evidence would imply, that whilst glial cells are not capable of vesicular release, they are able to mediate release by the reverse operation of electrogenic neurotransmitter carrier systems (Barres, 1991).

In the present study, 100 mM K\(^+\) was employed to provide a strong depolarising stimulus. The *in vitro* relative recoveries of amino acids by dialysis probes utilised in this study ranged between 40-50\% (see Section 2.2.2). Given the small size of K\(^+\) ions in comparison to the size of amino acids diffusing across the dialysis membrane, it is feasible that an estimated extracellular concentration of at least 40-50 mM K\(^+\), may have been achieved in the hippocampal extracellular space within the immediate vicinity of the probe. Such a stimulus is likely to have induced both Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent release of amino acids from both neurones and glia (Nicholls, 1989).

Elevated K\(^+\) gave rise to rapid, short-lasting peaks of release of the neurotransmitters aspartate, glutamate and GABA, and the non-transmitter amino acid taurine. Synaptosomal studies of K\(^+\)-stimulated release mechanisms (as with veratridine), have demonstrated Ca\(^{2+}\)-dependent release to be exhausted within a few minutes of beginning a constant depolarising stimulus, and subsequent efflux is considered to originate from cytoplasmic amino acid pools, with release being mediated by reversal of electrogenic Na\(^+\)-dependent amino acid carrier systems (Nicholls, 1989). Efficient inactivation of amino acid neurotransmitters by glial cells (Paulsen and Fonnum, 1984), and removal of extracellular [K\(^+\)] by glial high affinity K\(^+\) uptake sites (Barres,
Chapter 3: Acute effects of LTG on neurotransmitter efflux

1991; Kimelberg *et al.*, 1993), are responsible for terminating amino acid efflux. Glial cells have been shown to accumulate around the probe as part of the pathological response to tissue damage incurred during probe implantation (Lehmann *et al.*, 1983). Additionally, inactivation of glial cells with fluorocitrate (which selectively inhibits the aconitase in glial cells, thereby interfering with the tricarboxylic acid cycle in these cells), has been demonstrated to temporarily increase the Ca\(^{2+}\)-dependent, K\(^+\)-stimulated release of glutamate in striatal dialysates (Paulsen and Fonnum, 1989). Furthermore, what effect, a reduction in the NaCl content (125 mM to 27.5 mM) of the stimulating infusing solution (to maintain osmolarity) may have had on the extracellular [Na\(^+\)], which is necessary for Na\(^+\)-dependent, high affinity, amino acid uptake mechanisms, is unclear. Extracellular [Na\(^+\)] is unlikely to have been significantly reduced, since not all Na\(^+\) ions would have crossed the dialysis membrane, and influx from surrounding brain areas would probably have offset any considerable reduction. However, it remains possible that a decrease in external [Na\(^+\)] may have been sufficient to reduce uptake and thereby add to the increase in neurotransmitter efflux. In contrast, glutamine levels fell, consistent with its precursor role for glutamate and GABA synthesis (Tossman *et al.*, 1986; Young and Bradford, 1986). Generally, K\(^+\)-evoked S2 levels were comparable to those achieved during S1, suggesting sufficient time between stimulating pulses for restoration of cytoplasmic [Ca\(^{2+}\)] and/or replenishment of releasable transmitter pools (Nicholls, 1989). LTG did not alter K\(^+\)-evoked amino acid efflux, suggesting that it does not affect Ca\(^{2+}\)-dependent neurotransmitter release mechanisms.

Similarly, rapid, short-lasting elevations in dopamine and 5-HT efflux, concomitant with reductions in the levels of their metabolites, evoked by high K\(^+\), were observed. These effects are consistent with activation of Ca\(^{2+}\) channels by elevated K\(^+\), thereby stimulating the predominantly Ca\(^{2+}\)-dependent, exocytotic release of dopamine and 5-HT, which are then efficiently removed from the extracellular space by re-uptake mechanisms (Badoer *et al.*, 1989; Matos *et al.*, 1990; Sharp *et al.*, 1990). Reductions in the extracellular levels of DOPAC, HVA and 5-HIAA most probably reflect a depolarisation-evoked decline in the metabolism of dopamine and 5-HT, thereby

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preserving neurotransmitters to replace vesicular stores depleted by elevated K+ (Sharp et al., 1989; Crespi et al., 1990; Sorkin et al., 1991). The responses to 5-HIAA must be treated with caution however, given the experimental conditions used. The presence of the 5-HT uptake inhibitor, citalopram, in the infusion medium, to bring basal 5-HT to detectable levels, will have of course limited the amount of 5-HT available for metabolism in 5-HT neurones. These effects were also not altered by LTG. The total lack of effect upon both K+-evoked amino acids and monoamines, would therefore suggest that LTG, at the doses administered, does not influence Na+-independent depolarisation-evoked neurotransmitter efflux.

The profiles of aspartate, glutamate, taurine and GABA efflux evoked by veratridine, were markedly different from those achieved with elevated K+, mainly because release did not revert to baseline levels as efficiently. An incomplete "washout" of veratridine, which is highly lipophilic, may have been responsible for producing such effects; these lingering effects thereby attenuating subsequent depolarisation by depleting releasable neurotransmitter pools. However, analysis of the samples immediately preceding S2, indicated that amino acid concentrations were returning to basal levels after veratridine removal (c.f. elevated K+ which is efficiently removed from the extracellular space by high-affinity uptake sites present in glial cells). Alternatively, since veratridine increases the intracellular [Na+], thereby reversing Na+-dependent amino acid uptake carriers (Nicholls, 1993), prolonged release of amino acids may be due to inefficient re-uptake into neurones and glia from the extracellular space (Schousboe, 1981; Fagg and Foster, 1983).

Proceeding S2, all amino acid efflux, with the exception of glutamate, was lower than that obtained during S1, representing incomplete replenishment of releasable (neuronal and metabolic) neurotransmitter pools. S2-evoked glutamate efflux initially rose sharply, before stabilising at a significantly elevated level. Since a similar effect was not observed during S1, would suggest less effective, or saturated re-uptake mechanisms. The profile of this effect is likely to represent the complex neurochemical effects produced by veratridine previously alluded to - a short-lasting, Ca2+-dependent
release, being masked by a long-lasting, sustained Ca$$^{2+}$$-independent, but Na$$^+$$-dependent efflux, mediated by reversed uptake (Dickie and Davies, 1993; Nicholls, 1993; Stier et al., 1996). In contrast, the release of GABA was not as prolonged as that of glutamate, although it too is removed into cells by Na$$^+$$-dependent uptake carriers (Attwell et al., 1993). Releasable pools of glutamate may be larger than those of GABA in the hippocampus, and/or perhaps glutamine (significantly attenuated by veratridine depolarisation) is preferentially being converted to glutamate, rather than GABA, under these conditions. In support of this, Paulsen and Fonnum (1989) postulated that transmitter glutamate may be preferentially synthesised when glutamine is in limited supply. However, whilst other neurotransmitters are removed from the synapse by enzymatic metabolism and uptake processes, extracellular excitatory amino acid concentrations are generally thought to be wholly regulated by uptake via Na$$^+$$-dependent high affinity transporters, with no extracellular metabolism (Dowd et al., 1996). These effects would suggest inefficient re-uptake to be responsible for the prolonged glutamate effect. Furthermore, Rothstein et al. (1996) have shown that loss of the glial glutamate transporters GLAST or GLT-1, but not the neuronal glutamate transporter EAAC1, to produce an elevation in glutamate levels in the striatum. These findings imply that ineffective functioning of the Na$$^+$$-dependent glial glutamate transporter in particular, may be responsible for the sustained rise in extracellular glutamate efflux. LTG dose-dependently inhibited the veratridine-evoked efflux of glutamate, aspartate, taurine and GABA in a significant (at 13.4 mg/kg LTG), but non-specific manner. It is likely that the differences in the magnitudes of these inhibitory effects are predominantly due to dissimilarities in the Na$$^+$$-dependency of release of different amino acids.

Therefore, it is possible that blockade of membrane voltage-dependent Na$$^+$$ conductances by LTG, may be capable of preventing the reversal of Na$$^+$$-dependent amino acid uptake carrier systems elicited by raised intracellular [Na$$^+$$]. Such an effect would thereby inhibit in part, Na$$^+$$-dependent (e.g. veratridine-evoked) amino acid efflux. In support of this, Riddall et al. (1993) have shown poor correlation between veratrine-evoked glutamate release and $^3$H-batrachotoxin binding, implying that the
effects of LTG to inhibit veratrine-stimulated glutamate release may not be due to a specific or exclusive interaction with Na\(^+\) channel site 2 alone, although blockade of Na\(^+\) flux is essential for this effect. Furthermore, binding studies have demonstrated that LTG does not affect the uptake of \(^{3}\)H-D-aspartate (Leach \textit{et al.}, 1995), suggesting that its effects on aspartate and glutamate efflux, are not mediated through a direct interaction with the Na\(^+\)-dependent glutamate uptake carrier which also transports aspartate (Nicholls, 1993).

In comparison, veratridine-evoked efflux of dopamine and 5-HT (and concomitant reductions in the levels of their metabolites), were not altered by LTG, further demonstrating a lack of effect on Ca\(^{2+}\)-dependent, exocytotic release mechanisms (Nicholls, 1993). Exocytotic release from vesicular amino acid stores is also likely to be contributable, certainly in part, for the remainder of veratridine-evoked amino acid efflux above baseline levels.

In summary, these \textit{in vivo} experimental findings demonstrate that LTG specifically inhibits the voltage-sensitive, Na\(^+\)-dependent (\textit{c.f.} K\(^+\)-evoked amino acid and monoamine efflux) efflux of amino acids but not monoamines, owing to the inherent anatomical differences in the Na\(^+\)-dependency of their release mechanisms. These effects are in accordance with the findings of \textit{in vitro} electrophysiological studies, which have demonstrated LTG to inhibit voltage-gated Na\(^+\)-channels in a use- and voltage-dependent manner, interacting with the inactivated conformation of the type IIa Na\(^+\) channel (Cheung \textit{et al.}, 1992; Lang \textit{et al.}, 1993; Lees and Leach, 1993; Xie \textit{et al.}, 1995). Effects of LTG on Ca\(^{2+}\) currents have been less consistently reported. LTG has been shown to inhibit a "presumptive" Ca\(^{2+}\) current in rat cortical cultures (Lees and Leach, 1993), whilst having no effect on either L- or T-type Ca\(^{2+}\) currents in whole cell clamped pituitary cells at therapeutic doses (Lang \textit{et al.}, 1993). However, more recent evidence has demonstrated LTG to inhibit N- and P-type Ca\(^{2+}\) currents in cortical neurones, also at doses capable of Na\(^+\) channel blockade (Stefani \textit{et al.}, 1996). The exact relevance of these effects to the mechanism of action of LTG remains to be elucidated.
Thus, Na\(^+\) channel modulation by LTG appears to be fundamental for reducing excitatory neurotransmission, as demonstrated in the present study. These LTG-mediated \textit{in vivo} (amino acids) neurochemical effects are consistent with the early \textit{in vitro} findings of Leach \textit{et al.} (1986), in which LTG blocked the veratrine-induced release of glutamate and aspartate, and less potently GABA, whilst having no effect on K\(^+\)-evoked amino acid release in rat brain cortical slices. Such findings thereby demonstrate properties which could be beneficial in the control of epileptic seizures.

The role of glutamate in the pathophysiology of epilepsy has been extensively studied, and has shown that excessive glutamatergic tone gives rise to epileptic seizures (Meldrum, 1994). During an epileptic seizure, the extracellular K\(^+\) concentration in the brain rises excessively (Meldrum, 1990). This would release more glutamate into the extracellular space by (a) depolarising neurones increasing their firing rate and increasing vesicular release of glutamate, and (b) promoting the release of glutamate by reversal of the plasma membrane glutamate uptake carrier. The resulting rise in glutamate concentration will depolarise neurones further, releasing more K\(^+\); this positive feedback tending to lead to a large rise in the extracellular glutamate concentration (Nicholls and Attwell, 1990). The magnitude of the LTG-evoked inhibition of glutamate efflux demonstrated in the present \textit{in vivo} experiments, would strongly suggest that LTG may be effective in keeping the extracellular glutamate concentration below levels that trigger neuronal hyperexcitability (Nicholls, 1993).

To conclude, these \textit{in vivo} data would suggest that at experimental anticonvulsant doses in rodents, the effects of LTG may be attributed to its ability to reduce predominantly pathologically-altered excitatory neurotransmission as a consequence of voltage-dependent Na\(^+\) channel blockade. (A reduction in GABA output should be pro-convulsant, but clearly was not, implying that overall excitatory : inhibitory neurotransmitter homeostasis was being maintained). This has the effect of stabilising excitable epileptic membranes, thereby preventing the pathological reversal of specifically, Na\(^+\)-dependent amino acid uptake systems, and subsequently attenuating the spread of intense neuronal excitation and therefore the generation of further epileptiform events.
CHAPTER 4

EFFECTS OF CHRONIC (UP TO 21 DAYS) LAMOTRIGINE ADMINISTRATION ON NEUROTRANSMITTER EFFLUX FROM THE RAT VENTRAL HIPPOCAMPUS: RELEVANCE TO CLINICAL ANTICONVULSANT EFFICACY
4.1 Introduction

Lamotrigine (Lamictal\textsuperscript{TM}) is a broad spectrum anticonvulsant (in both experimental animal models of epilepsy and in man), with a reduced side-effect profile compared to existing antiepileptic drugs (Upton, 1994). It is at present licensed as add-on therapy for the treatment of refractory partial and secondarily generalised seizures, with no evidence of anticonvulsant tolerance (Fitton and Goa, 1995; Messenheimer, 1995).

Current evidence suggests that LTG acts primarily via a use-dependent blockade of voltage-sensitive Na\textsuperscript{+} channels in their slow-inactivated state (Xie \textit{et al.}, 1995), to stabilise neuronal membranes, thereby inhibiting the Na\textsuperscript{+}- (but not Ca\textsuperscript{2+}-) dependent release of neurotransmitters, most notably glutamate (reviewed by Meldrum and Leach, 1994; Leach \textit{et al.}, 1995; Chapter 3 - this thesis). These actions are considered to be responsible for stabilising epileptic membranes, and preventing the subsequent pathological release of excitatory amino acids, and the generation of further epileptiform events. However, the ability of LTG to modulate neurotransmitter release is not confined to excitatory amino acids, since Na\textsuperscript{+}-dependent GABA release is also significantly inhibited (Leach \textit{et al.}, 1986; Chapter 3 - this thesis).

Whilst these effects of LTG are fundamental for its mechanism of action, comparatively little is known of the nature of these neurochemical changes \textit{in vivo}, and how they may be modified following chronic treatment with LTG. Since LTG is prescribed for prolonged usage, repeated exposures to the drug may uncover properties that suppress seizures by inducing slow changes (\textit{e.g.} ionic distribution, endocrine and metabolic function, or gene expression to modify specific receptor densities) and/or biochemical abnormalities that might occur only after chronic administration. Consequently, how prolonged neurochemical changes following chronic LTG-treatment may influence overall clinical anticonvulsant efficacy remains unclear. Such effects are important, since a reduction in GABA release may produce pro-convulsant effects, thereby limiting the overall anticonvulsant potential of LTG. Furthermore, the relationship between LTG dosage, plasma/ brain/ ECF concentrations, neurochemical effects and anticonvulsant efficacy remain to be elucidated.
Plasma levels of antiepileptic drugs have required that these levels reflect the drug concentration at the neuronal sites of action. Many drugs, such as carbamazepine and phenytoin, are believed to exert an effect at the neuronal membrane of cells within and surrounding the seizure focus (Rogawski and Porter, 1990). However, it remains unclear exactly how much drug is available for action at the neuronal membrane. This is relevant since LTG has a primary action at the Na⁺ channel. The traditional assumption is that the available drug mirrors the unbound plasma or serum concentration (Greenblatt et al., 1982). However, several investigators have challenged this concept, and argued that drug entry into and exit from the brain are not symmetric processes (Cornford and Oldendorf, 1986; Jones et al., 1988). Microdialysis offers an opportunity to measure antiepileptic drug concentrations in the brain and study the relationship between blood and ECF concentrations.

In the present study, these issues have been addressed using intracerebral microdialysis, to sample the extracellular environment of rat ventral hippocampal tissue for amino acids and monoamines, during and up to a 21 days treatment period with LTG. In addition, differences in neurotransmitter efflux evoked by the voltage-dependent Na⁺ channel activator veratridine (50 μM), between treated and control animals, were assessed. Whole brain tissue, plasma and dialysate LTG concentrations were also measured, to determine the relationship between ECF and unbound plasma LTG concentrations, thereby demonstrating whether the measured LTG concentration in plasma is an appropriate and reliable indicator of the active brain drug concentration.

These findings are discussed in terms of a relationship between LTG concentrations in brain, plasma and ECF with neurochemical effects and clinical anticonvulsant efficacy.
4.2 Methods

4.2.1 Experimental protocol

Two randomly selected groups of rats (n = 30/group), were initially group housed and their body weights recorded daily throughout the period of study. A fixed dose, fixed time schedule treatment regimen was established such that all animals in the test groups received LTG twice daily (6.70 mg/kg base i.p. - single dose) at approximately 08.00 h and 20.00 h. Since the half-life of LTG is between 12-15 h in rats (Parsons et al., 1995), a 12 h dosing interval was employed to maintain minimal oscillations between maximum and minimum concentrations during drug administration. Control animals received the appropriate vehicle (distilled water) injections each day at the equivalent times. Both test and control rats were sub-grouped into sets of six animals on day one (i.e. 5 x n = 6 test, 5 x n = 6 control), and between 13.00 h and 18.00 h (day 1), a test and control sub-group were implanted with microdialysis probes into the ventral hippocampus, as previously described (see Section 2.2.3). All animals were allowed to recover from anaesthesia, and administered either LTG or vehicle at 20.00 h. On the following day, all animals received their appropriate treatments at 08.00 h.

Microdialysis was always commenced at approximately 13.00 h. After the standard 60 min. stabilisation period (which was used for determination of dialysate LTG concentration), five consecutive 60 min. dialysate samples were collected from each rat. Between dialysate samples 2 and 3, a 30 min. sample was collected, and during this period all rats received an infusion of veratridine (50 μM) through the probe, before returning to the original dialysis medium.

4.2.2 Biochemical analysis

All dialysate samples were divided into two equal portions and analysed for selected amino acids and monoamines using HPLC-FD or HPLC-ED respectively, as previously described (see Section 2.3).
The entire microdialysis procedure was repeated for animals injected for 4, 7, 14 and 21 days with twice daily LTG- or vehicle-treatments. This protocol was also repeated at the lower dose of 2.01 mg/kg LTG base (single dose) at 21 days only.

4.2.3 Determination of plasma, brain and microdialysate LTG concentrations

Following the completion of microdialysis experiments, rats were terminally anaesthetised with CO₂, before the collection of blood via cardiac puncture, into 10 ml heparinised tubes. The blood was centrifuged at 13,000 g for 10 min. in a MSE microcentaur to obtain plasma. Additionally, brains were rapidly dissected out and stored at -80 °C, until subsequent analysis. Whole brain, microdialysate and plasma LTG concentrations were analysed by HPLC-MS as previously described (see Section 2.4).

4.2.4 LTG in vitro relative probe recovery

To determine the relevance of the extracellular LTG concentrations attained, it was necessary to calculate the relative probe recovery of LTG.

LTG was dissolved in double distilled, deionised water, to a final concentration of 1 μM. Probes (n=4) were suspended in this stirred bathing medium (37 °C) and infused with aCSF at a flow rate of 0.5 μl/min., and after a 1 h equilibration period, four consecutive 30 min. samples were collected. In all cases, aCSF was delivered to the probe inlet tubing via a 30 cm length of pp 10 tubing fitted to a gas-tight 500 μl microsyringe.

4.2.5 Statistical analysis

Basal levels of amino acids and monoamine neurotransmitters and their metabolites at each time-point are presented in tabular form, with absolute concentrations shown to illustrate neurochemical differences between treatments during chronic
administration. Neurotransmitter time-course effects at each time-point are presented in bar graph form, with all values recorded as a percentage of the normalised mean basal concentrations achieved in the respective control groups. This scheme was adopted to counteract the large amount of variation in neurotransmitter concentrations between individual animals, whilst preserving any neurochemical differences between the two treatment groups. The Mann-Whitney U test was used to analyse for statistically significant differences between baseline and peak neurotransmitter concentrations in control and treatment groups at each time-point. The time-course for neurotransmitter efflux within each treatment group, was analysed for changes relative to baseline using ANOVA with repeated measures; p<0.05 was regarded as statistically significant.
4.3 Results

4.3.1 Body weights

There were no significant differences in daily body weights between LTG- (2.01 and 6.70 mg/kg / 2 x daily) and vehicle-treated animals.

Fig. 4(a): Effects of chronic (up to 21 days) LTG- (2.01 and 6.70 mg/kg) or vehicle- (distilled water) administration on terminal body weights'. Bars are means ± s.e. mean (n=6/group). 'Body weights represent those on day of surgery.

4.3.2 Behaviour

There were no apparent gross behavioural differences (e.g. sniffing, exploration, feeding, drinking, grooming) between LTG- and vehicle-treatments. All LTG-treated animals survived anaesthesia and surgery, with no observed differences between controls and drug-treated animals.
4.3.3 Effects of chronic (up to 21 days) LTG- (2.01 and 6.70 mg/kg/2 x daily) administration on plasma, whole brain and microdialysate LTG concentrations.

Using a fixed dose, fixed time schedule drug-administration regimen, plasma, brain and microdialysate LTG concentrations increased over the first few days of drug treatment (up to 4 days), before reaching steady-state (see Fig. 4(b)). LTG concentrations were then maintained at this stabilised level for the remaining (up to) 17 days. Typical representative selection reaction monitoring (SRM) traces and internal standard and LTG spectra obtained by HPLC-MS, for the determination of LTG concentrations, are illustrated in Figs. 4(c)-(e).

4.3.3.1 Steady-state plasma, brain and microdialysate LTG concentrations: Relationship to dosage

LTG exhibited linear kinetics; the steady-state plasma, brain and microdialysate LTG concentrations were directly proportional to the dose of LTG administered (see Fig. 4(b)).

4.3.4 LTG in vitro relative probe recovery

The concentrations of LTG recovered via the microdialysis probe did not differ significantly between probes, and furthermore remained constant between consecutive 30 min. sample periods.

The mean [LTG] recovered was 0.10 ± 0.0070 µg/ml.

.: If probes were placed into a 1µM LTG (in distilled water) stock solution (flow rate = 0.5µl/min.) and 0.1µg/ml LTG was recovered (382 mg/ml ≡ 1 M) then, 0.1µg/ml ≡ 0.261 x 10⁻⁶ M, and recovery = 26%.
Chapter 4: Effects of chronic LTG administration

LTG concentrations in rat (A) ECF, (B) plasma and (C) brain samples at different time-points (up to 21 days) following a chronic administration regimen (2.01 (■) and 6.70 (●) mg/kg/2x daily).

Means ± s.e.mean are shown (n=6). *not corrected for in vitro recovery.
Chapter 4: Effects of chronic LTG administration

Fig. 4(c): Typical representative selection reaction monitoring traces.

Injections of (A) control rat brain homogenate, (B) control rat brain homogenate spiked with LTG at a concentration equivalent to 0.4 g/g of brain and (C) a brain sample from day 2 of chronic LTG- (6.70 mg/kg/2 x daily) administration.
Fig. 4(d): Typical representative heated nebuliser spectra.
(A) Internal standard, compound 725C78 and (B) collisional activation spectrum of the protonated molecule (m/z 218) of compound 725C78.
Chapter 4: Effects of chronic LTG administration

Fig. 4(e): Typical representative heated nebuliser spectra. (A) lamotrigine and (B) collisional activation spectrum of the protonated molecule (m/z 256) of lamotrigine.
Chapter 4: Effects of chronic LTG administration

4.3.4.1 Relationship between plasma and ECF LTG concentrations

Parsons et al. (1995) have reported LTG to be 54% plasma protein bound, if total steady-state plasma [LTG] = 7.43 ± 0.31 µg/ml, then free plasma [LTG] = 3.42 ± 0.14 µg/ml,

and if ECF steady-state [LTG] = 0.726 ± 0.04 µg/ml, then ECF [LTG] (corrected for in vitro recovery) = 2.79 ± 0.15 µg/ml,

∴ [LTG] ECF : plasma = 0.82.

4.3.5 Effects of chronic (up to 21 days) LTG-administration on basal and veratridine-evoked amino acid efflux

4.3.5.1 Basal amino acid efflux

The effects of chronic (up to 21 days) LTG-administration (2.01 and 6.70 mg/kg) on the basal concentrations of selected amino acids in the ventral hippocampus are presented in Table 4(a). All values were calculated from the mean values in the two samples collected prior to veratridine stimulation. Basal amino acid concentrations were not statistically significantly different between LTG- and vehicle-treatments at any time-point up to and including day 21 (Mann-Whitney U test, p < 0.05). However, not all basal amino acid concentrations remained constant with respect to time. Basal aspartate concentrations on day 2 were approximately 2-fold higher than those achieved on day 4 and at subsequent time-points, which were all comparable. In contrast, only on day 4, glutamate concentrations were significantly elevated (approximately 2-fold) above day 2 values (ANOVA followed by Dunnett's t-test for multiple comparisons, p < 0.05). Glutamine and taurine concentrations also fluctuated between time-points.
<table>
<thead>
<tr>
<th>AMINO ACIDS</th>
<th>DAY</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>14</th>
<th>21</th>
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<tbody>
<tr>
<td><strong>ASP</strong></td>
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<tr>
<td>C</td>
<td></td>
<td>2.09 ± 0.23</td>
<td>*0.96 ± 0.13</td>
<td>*0.90 ± 0.18</td>
<td>*0.90 ± 0.18</td>
<td>*0.93 ± 0.13</td>
</tr>
<tr>
<td>T</td>
<td></td>
<td>2.08 ± 0.13</td>
<td>*1.16 ± 0.12</td>
<td>*1.07 ± 0.37</td>
<td>*0.88 ± 0.0</td>
<td>*0.87 ± 0.04</td>
</tr>
</tbody>
</table>

| **GLU**     |     |   |   |   |    |    |
| C           |     | 34.59 ± 4.61 | *69.57 ± 12.40 | 31.72 ± 3.19 | 33.79 ± 4.44 | 32.80 ± 1.20 |
| T           |     | 38.25 ± 3.75 | *65.83 ± 3.97 | 29.17 ± 6.64 | 37.73 ± 5.56 | 31.70 ± 7.00 |

| **GLN**     |     |   |   |   |    |    |
| C           |     | 40.20 ± 6.71 | 51.74 ± 12.07 | *31.47 ± 0.33 | 34.79 ± 4.25 | 45.60 ± 8.61 |
| T           |     | 35.95 ± 2.65 | 48.48 ± 26.68 | 33.13 ± 0.63 | 44.85 ± 9.93 | 41.87 ± 6.93 |

| **TAU**     |     |   |   |   |    |    |
| C           |     | 126 ± 20.50 | *77.13 ± 6.00 | *211.1 ± 19.4 | 136 ± 18.21 | *62.11 ± 8.00 |
| T           |     | 102 ± 28.50 | *69.70 ± 0.17 | *189 ± 15.50 | *154 ± 22.44 | *69.88 ± 3.20 |

| **GABA**    |     |   |   |   |    |    |
| C           |     | 0.52 ± 0.05 | 0.56 ± 0.01 | 0.475 ± 0.04 | 0.58 ± 0.03 | 0.59 ± 0.03 |
| T           |     | 0.54 ± 0.01 | 0.59 ± 0.31 | 0.43 ± 0.07 | 0.62 ± 0.08 | 0.61 ± 0.07 |

Table 4(a): Basal extracellular amino acid concentrations in the rat ventral hippocampus during chronic (up to 21 days) LTG- (2.01 and 6.70 mg/kg/ 2x daily i.p.) (T) or vehicle- (distilled water) (C) administration.

All values are mean pmols/15μl ± s.e.mean (n=5-6/group), *p<0.05, represents a significant difference from day 2 amino acid concentrations within treatment groups (ANOVA followed by Dunnett's t-test for multiple comparisons); *Concentrations represented in parentheses.
4.3.5.2 Veratridine-evoked amino acid efflux

Veratridine infusion (50 μM) via the microdialysis probe was accompanied by behavioural activation as previously described (see Section 3.3.1.2.1) in control animals at all time-points. These behavioural effects were reversible, out lasting the period of stimulation by approximately 30 min. However, at the higher dose of LTG (6.70 mg/kg) animals were less active, whilst no changes in gross behaviour were noticeable in response to veratridine infusion in animals treated with 2.01 mg/kg LTG in comparison to vehicle-treated animals.

Figs. 4(f)-(j) are graphical time-based representations of both basal and veratridine-stimulated amino acid dialysate contents following 2, 4, 7, 14 and 21 days of LTG (2.01 and 6.70 mg/kg/ 2 x daily) administration.

4.3.5.2.1 Aspartate

Veratridine caused a significant increase in aspartate efflux, beginning in the same sample as its addition to the infusing medium; maximal concentrations were reached in the second sample period proceeding veratridine infusion (2-3 fold above baseline levels), and returned to control levels within the next 60 min. collection period. LTG (2.01 and 6.70 mg/kg) dose- and time-dependently (up to the accomplishment of steady-state brain and plasma levels) attenuated the veratridine-evoked efflux of aspartate maximally to 27 ± 10% and significantly below baseline levels to 97 ± 36% at 2.01 and 6.70 mg/kg respectively at 21 days. However, the magnitude of the LTG-induced (6.70 mg/kg) inhibition of veratridine-evoked aspartate efflux achieved on days 7 and 14, was comparable (94 ± 23% and 96 ± 31% respectively) to that achieved on day 21. At 2.01 mg/kg LTG, and on days 2, 4 and 7 of 6.70 mg/kg LTG treatment, aspartate efflux had returned to baseline levels within the second sample period after the removal of veratridine from the infusing medium, but on days 14 and 21 aspartate efflux remained significantly depressed below baseline levels for the entire duration of sampling (see Fig. 4(f)).
Fig. 4(f): Effects of chronic (up to 21 days) LTG- (2.01 and 6.70 mg/kg/2 x daily) or vehicle- (distilled water) administration on basal and veratridine-evoked (50 μM) aspartate efflux from the rat ventral hippocampus.

The box marked "V" denotes the time-period in which veratridine was infused through the microdialysis probe. Means ± s.e. mean are shown (n=5-6/group); *p<0.05, **p<0.01, represents a significant difference from vehicle (Mann-Whitney U test).
4.3.5.2.2 Glutamate

A 1-2 fold increase in glutamate efflux was elicited by veratridine infusion, occurring maximally in the first sample after veratridine removal, and returning towards baseline levels thereafter. LTG dose-dependently inhibited veratridine-evoked glutamate efflux by maximally $39 \pm 25\%$ and $98 \pm 28\%$ at 2.01 and 6.70 mg/kg respectively at 21 days. In contrast to the reduction in aspartate efflux, the magnitude by which veratridine-elicited glutamate efflux was attenuated by LTG (6.70 mg/kg) was comparable, and greater than 90\%, at all time-points. The duration of the inhibitory effect was variable between successive time-points up to 7 days at 6.70 mg/kg LTG, at which point and successive time-points, there was no significant difference in outflow compared to control levels in the last sample period (see Fig. 4(g)).

4.3.5.2.3 Glutamine

Veratridine infusion significantly reduced glutamine efflux to approximately 25\% of baseline levels. 2.01 mg/kg LTG treatment had no significant effect upon the profile of veratridine-evoked glutamine efflux. At 6.70 mg/kg LTG however, glutamine efflux was significantly attenuated up to day 4 ($> 95\%$ reduction of baseline levels). In contrast on day 7, the veratridine-evoked inhibitory effect was partially reversed (reduced by $39 \pm 19\%$ of baseline levels), such that glutamine efflux remained significantly elevated ($90 \pm 34\%$) above the attenuated control values. In comparison, on days 14 and 21, there were no significant differences between treatments in the magnitude of the veratridine-evoked inhibitory effects (see Fig. 4(h)).
Fig. 4(g): Effects of chronic (up to 21 days) LTG- (2.01 and 6.70 mg/kg/2 x daily) or vehicle- (distilled water) administration on basal and veratridine-evoked (50 μM) glutamate efflux from the rat ventral hippocampus. The box marked “V” denotes the time-period in which veratridine was infused through the microdialysis probe. Means ± s.e. mean are shown (n=5-6/group); *p<0.05, **p<0.01, represents a significant difference from vehicle (Mann-Whitney U test).
Fig. 4(h): Effects of chronic (up to 21 days) LTG- (2.01 and 6.70 mg/kg/ 2 x daily) or vehicle- (distilled water) administration on basal and veratridine-evoked (50 μM) glutamine efflux from the rat ventral hippocampus.

The box marked "V" denotes the time-period in which veratridine was infused through the microdialysis probe. Means ± s.e. mean are shown (n=5-6/group); *p<0.05 represents a significant difference from vehicle (Mann-Whitney U test).
Chapter 4: Effects of chronic LTG administration

4.3.5.2.4 Taurine

Veratridine elicited a 1-2 fold increase in taurine efflux above baseline levels in control animals. LTG (2.01 mg/kg) did not significantly alter taurine efflux in response to veratridine infusion - the effect was comparable to the control value. In contrast, extracellular taurine levels at 6.70 mg/kg LTG were variable between time-points. The efflux of taurine was significantly attenuated on days 2 (by 49 ± 12 %) and 7 (by 64 ± 15 %), with no change in veratridine-evoked efflux in comparison to control values at the other time-points. However, veratridine-evoked taurine efflux did remain significantly elevated above equivalent control levels after veratridine removal on days 14 (by 33 ± 10 %) and 21 (by 32 ± 9 %) (see Fig. 4(i)).

4.3.5.2.5 GABA

Veratridine significantly increased the control basal extracellular GABA concentration between 10-20 fold. LTG dose-dependently and significantly attenuated the depolarisation-induced efflux by 46 ± 19 % and 87 ± 34 % of control values, at 2.01 and 6.70 mg/kg LTG respectively at 21 days. As with glutamate efflux, the profile of the LTG-induced inhibition of GABA efflux, was comparable to the day 21 value at all time-points. On days 2 and 4, veratridine-evoked GABA efflux had returned to baseline levels in the last sample period in both treatment groups. However, at subsequent time-points, GABA efflux in all, except the group treated with 6.70 mg/kg LTG, was significantly elevated above baseline levels for the duration of sampling (ANOVA followed by Dunnett’s t-test for multiple comparisons) (see Fig. 4(j)).
Fig. 4(i): Effects of chronic (up to 21 days) LTG- (2.01 and 6.70 mg/kg/2 x daily) or vehicle- (distilled water) administration on basal and veratridine-evoked (50 μM) taurine efflux from the rat ventral hippocampus.

The box marked "V" denotes the time-period in which veratridine was infused through the microdialysis probe. Means ± s.e. mean are shown (n=5-6/group); *p<0.05, **p<0.01, represents a significant difference from vehicle (Mann-Whitney U test).
Fig. 4(j): Effects of chronic (up to 21 days) LTG- (2.01 and 6.70 mg/kg/2 x daily) or vehicle- (distilled water) administration on basal and veratridine-evoked (50 μM) GABA efflux from the rat ventral hippocampus.

The box marked "V" denotes the time-period in which veratridine was infused through the microdialysis probe. Means ± s.e. mean are shown (n=5-6/group); *p<0.05, **p<0.01, represents a significant difference from vehicle (Mann-Whitney U test).
4.3.6 Effects of chronic (up to 21 days) LTG-administration on basal and veratridine-evoked monoamine efflux

4.3.6.1 Basal monoamine efflux

The effects of chronic (up to 21 days) LTG administration (2.01 and 6.70 mg/kg) on basal dopamine, DOPAC, HVA and 5-HT and 5-HIAA concentrations in the ventral hippocampus are presented in Table 4(b). All values were calculated from the mean values in the two samples collected prior to veratridine stimulation.

There were no statistically significant differences in basal dopamine concentrations between LTG- and vehicle-treatments at any time-point. However, basal dopamine efflux was elevated non-significantly on days 2 and 4, the magnitude of this effect being most pronounced on day 4. The evident trend towards an elevation in basal dopamine efflux existing up to day 7 of chronic 6.70 mg/kg LTG-treatment, was reflected in a similar tendency in the ability of LTG to modify extracellular levels of DOPAC and HVA. Basal DOPAC efflux was non-significantly raised on days 4 and 7 of treatment. By day 14, concentrations had been restored to equivalent control values. HVA efflux was also non-significantly elevated on days 2 and 4 of chronic treatment (6.70 mg/kg LTG), with no change compared to control values at successive time-points.

There were also no statistically significant differences in basal 5-HT concentrations between LTG- (2.01 and 6.70 mg/kg) and vehicle-treatments at any time-point. However, basal 5-HT concentrations were elevated non-significantly in comparison to the corresponding control values on days 2, 14 and 21 of chronic LTG- (6.70 mg/kg) treatment. In contrast, there was no significant change in the basal efflux of 5-HIAA compared to equivalent control values at any time-point.
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<td></td>
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<td>C</td>
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<td>0.051 ± 0.017</td>
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<td>T</td>
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<td>0.208 ± 0.161</td>
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<tr>
<td>C</td>
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<td></td>
</tr>
<tr>
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<td>0.045 ± 0.012</td>
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<td>0.0518 ± 0.006</td>
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<td>0.127 ± 0.076</td>
<td>0.046 ± 0.011</td>
<td>0.071 ± 0.027</td>
<td>0.056 ± 0.012 (0.054 ± 0.016)</td>
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<tr>
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<td>0.076 ± 0.019</td>
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<tr>
<td>5-HIAA</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>4.92 ± 0.34</td>
<td>3.90 ± 0.48</td>
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<td>2.021 ± 0.42</td>
<td>3.47 ± 0.016 (3.17 ± 0.021)</td>
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Table 4(b): Basal extracellular monoamine concentrations in the rat ventral hippocampus during chronic (up to 21 days) LTG- (\(0.01\) and \(6.70\) mg/kg/ 2 x daily i.p.) (T) or vehicle-(distilled water) (C) administration.

All values are mean pmols/ 15μl ± s.e.mean (n=5-6/group). Concentrations represented in parentheses.
4.3.6.2 Veratridine-evoked monoamine efflux

Infusion of veratridine (50 μM; n=5-6/group) was associated with behavioural activation, elevations in dopamine (300-800 %) and 5-HT (300-800 %), with a decline in the concentrations of their respective metabolites, DOPAC (50-60 %) and HVA (10 %) which then rose above baseline concentrations, and 5-HIAA (30-40 %) of basal levels. The maximal effects of stimulation-evoked dopamine and 5-HT efflux were observed either in the same sample as that of veratridine infusion, or that immediately proceeding it, thereafter returning towards baseline concentrations. LTG-administration (2.01 and 6.70 mg/kg) had no statistically significant effects on veratridine-evoked dopamine, DOPAC and HVA or 5-HT and 5-HIAA efflux compared to corresponding control values at any time-point up to 21 days (see Figs. 4(k)-(o)).
Fig. 4(k): Effects of chronic (up to 21 days) LTG- (2.01 and 6.70 mg/kg/2 x daily) or vehicle- (distilled water) administration on basal and veratridine-evoked (50 μM) dopamine efflux from the rat ventral hippocampus.

The box marked "V" denotes the time-period in which veratridine was infused through the microdialysis probe.
Fig. 4(i): Effects of chronic (up to 21 days) LTG- (2.01 and 6.70 mg/kg/ 2 x daily) or vehicle- (distilled water) administration on basal and veratridine-evoked (50 μM) DOPAC efflux from the rat ventral hippocampus.

The box marked "V" denotes the time-period in which veratridine was infused through the microdialysis probe.
Fig. 4(m): Effects of chronic (up to 21 days) LTG- (2.01 and 6.70 mg/kg/2 x daily) or vehicle- (distilled water) administration on basal and veratridine-evoked (50 μM) HVA efflux from the rat ventral hippocampus.

The box marked "V" denotes the time-period in which veratridine was infused through the microdialysis probe.
Fig. 4(n): Effects of chronic (up to 21 days) LTG- (2.01 and 6.70 mg/kg/2 x daily) or vehicle- (distilled water) administration on basal and veratridine-evoked (50 μM) 5-HT efflux from the rat ventral hippocampus.

The box marked "V" denotes the time-period in which veratridine was infused through the microdialysis probe.
Fig. 4(a): Effects of chronic (up to 21 days) LTG- (2.01 and 6.70 mg/kg/2 x daily) or vehicle- (distilled water) administration on basal and veratridine-evoked (50 μM) 5-HIAA efflux from the rat ventral hippocampus.

The box marked "V" denotes the time-period in which veratridine was infused through the microdialysis probe.
4.4 Discussion

The use of many agents for the long-term treatment of epilepsy, has been greatly restricted by their ability to induce anticonvulsant tolerance and dependence e.g. benzodiazepines and barbiturates (Haigh and Feely, 1988). Anticonvulsant tolerance has also been shown to develop with other antiepileptic drugs including carbamazepine (Frey, 1987, 1986). Additionally, the therapeutic effectiveness of traditional antiepileptic agents has in general, been limited by their narrow therapeutic index (Richens, 1991). This has led to the development of a new generation of potential antiepileptic agents for the management of this chronic disorder, which include LTG. Both animal and human studies have shown LTG to exhibit a broad spectrum of activity with a reduced side-effect profile, compared to established antiepileptic drugs (Upton, 1994).

Since LTG is prescribed for prolonged usage, the present study has examined the nature and profile of the neurochemical effects on amino acids and monoamines during chronic (up to 21 days), twice daily LTG-administration, together with determination of the brain, plasma and extracellular fluid LTG concentrations. The relevance of the observed effects of LTG has been considered in relation to human therapeutic levels and anticonvulsant efficacy.

4.4.1 Methodological considerations

The graphical data was depicted in such a manner to counteract the large amount of variation in the absolute quantities of neurotransmitters existing between animals and secondly, that which existed between repeat experiments. Furthermore, individual nutritional status, and of course, slight variations in microdialysis probe placements, relative probe recoveries and reaction to the drug treatment itself, will have all contributed to the sources of variation, resulting in the relatively large standard errors prevalent in this study.
Chapter 4: Effects of chronic LTG administration

In spite of these difficulties, the findings reported here demonstrate that LTG differentially alters neurotransmitter (and their metabolites) efflux, during chronic treatment in rats without significant adverse changes in weight or gross behaviour.

4.4.2 Neurochemical effects during chronic (up to 21 days) LTG-administration

Chronic LTG-administration (2.01 and 6.70 mg/kg i.p. twice daily/21 days) specifically, dose-dependently and statistically significantly attenuated veratridine-evoked (Na⁺-dependent) amino acid, but not monoamine, efflux. As previously alluded to in Chapter 3, the differences in the magnitudes of these inhibitory effects, may predominantly be due to dissimilarities in the release Na⁺-dependency of various neurotransmitters. LTG was able to completely inhibit the veratridine-evoked release of the excitatory amino acids glutamate and aspartate, whilst also significantly attenuating the stimulated release of GABA at the higher dose of 6.70 mg/kg LTG. The veratridine-evoked reduction in glutamine efflux was also partially reversed at day 7 onwards. These findings demonstrate that LTG may be capable of attenuating, predominantly the excessive or increased, pathological release of excitatory amino acids as may occur in epilepsy, thereby maintaining overall excitatory:inhibitory neurotransmitter homeostasis, at the doses administered. The magnitude of the inhibitory effects at 21 days was comparable to that sustained and maintained at the previous time-points investigated during and up to 14 days, with a complete absence of neurochemical tolerance. Typically, adaptive changes did not occur, instead the neurochemical responses remained uniform with respect to time once steady-state LTG levels had been achieved. In contrast, but consistent with the findings of acute LTG administration (Chapter 3), the veratridine-evoked efflux of monoamines, together with basal amino acid and monoamine concentrations remained unaltered, suggesting that chronically, LTG does not significantly influence net neurotransmitter efflux due to release (Ca²⁺-dependent exocytosis), re-uptake and/or metabolism/degradation. Both doses of LTG used were known to be within the effective anticonvulsant range as reported in early animal studies, which demonstrated the peak effect (1h) ED₅₀ for the inhibition of MES-induced seizures in rats to be 3.60 mg/kg i.p. Additionally,
tolerance to this anticonvulsant action of LTG was not observed following administration for up to 28 days in rats, with electroshock applications once weekly to determine ED\textsubscript{50} values (Miller et al., 1986).

These results are in agreement with the previous early \textit{in vitro} neurochemical findings of Leach \textit{et al.} (1986), in which LTG inhibited the release of amino acids evoked by veratrine, but had no significant effects on spontaneous or K\textsuperscript+-evoked amino acid release, and more recently also accord with acute \textit{in vivo} findings (Chapter 3 - this thesis). These data are thereby consistent with previous reports, which have suggested that interference of Na\textsuperscript{+} flux across neuronal membranes, may be the basis of the anticonvulsant effects of LTG (Cheung \textit{et al.}, 1992; Xie \textit{et al.}, 1995). By altering tissue sodium homeostasis by use-dependent blockade of voltage-sensitive Na\textsuperscript{+} channels, LTG deprives the cell of cytosolic Na\textsuperscript{+}, consequently preserving the normal Na\textsuperscript{+} gradient. This effect serves to prevent high-frequency neuronal firing resulting in less, pathologically-altered amino acid release, and secondly by maintaining the intracellular and extracellular Na\textsuperscript{+} gradient, the inward movement of amino acid neurotransmitters (via the Na\textsuperscript{+}-coupled amino acid uptake transporters).

\textbf{4.4.3 Association between LTG concentration and neurochemical effects}

Whilst LTG is capable of reducing veratridine-evoked amino acid neurotransmitter efflux both \textit{in vitro} and \textit{in vivo}, the relevance of these inhibitory effects to its anticonvulsant efficacy remain unclear.

Early animal studies, identified rat plasma levels of LTG of approximately 3 \textmu g/ml to provide protection (4 x ED\textsubscript{50}) from the maximum electroshock. These results thereby provided a plasma concentration of LTG that needed to be achieved in early human studies and was used, proving to be effective in clinical trials (Peck, 1992; Wolf, 1992; Burstein, 1995), with no evidence of anticonvulsant tolerance (Schachter, 1995).

In this study, plasma concentrations of LTG increased over the first few days of
administration, with steady-state levels being achieved by day 4. Stable plasma concentrations then persisted for the remaining (up to) 17 days of the study, with no apparent "falling off" of plasma LTG concentrations. This suggests that if autoinduction was occurring, the effects were manifest in the early stages of treatment when the plasma levels of LTG were rising to steady-state. A similar profile has been clinically demonstrated with LTG monotherapy (Richens, 1992).

The therapeutic concentration range of LTG has yet to be definitively established, since LTG is at present administered mainly in combination with other antiepileptic drugs which may have differing effects on therapeutic LTG levels. Early studies have suggested that despite the lack of correlation between serum LTG concentrations and effect, a reduction in EEG spike counts was greatest at serum LTG concentrations of 1-3 µg/ml. However, patients have tolerated concentrations up to 20 µg/ml with benefit and without clinical toxicity (Richens, 1995a). Therefore, considering that plasma LTG concentrations achieved in the present study were within this clinically effective anticonvulsant range, the observed magnitude by which veratridine-evoked excitatory amino acid neurotransmitter efflux was attenuated by chronic LTG-treatment, may be sufficient to maintain glutamate and aspartate levels below those which are deleterious to neurones, such effects thereby contributing to the overall anticonvulsant efficacy of LTG.

Underlying the use of blood level determinations of antiepileptic drugs, is the assumption that these levels are related in some way to efficacy (Rambeck et al., 1990). Furthermore, the basis for the interpretation of plasma antiepileptic drug concentrations, requires that these levels reflect the drug concentration at the neuronal sites of action. This is of particular importance for agents such as LTG, which exert an effect at the neuronal membrane of cells within, and surrounding, the seizure focus. Therefore, how much of the drug is available for action at the neuronal membrane, and whether this is representative of the unbound plasma, presumed active drug concentration, remains unclear. In the present study, dialysate LTG levels were approximately 3 µg/ml and 8 µg/ml respectively at 2.01 and 6.70 mg/kg twice daily.
administration at 21 days, and thereby illustrate the ability of microdialysis to measure antiepileptic drug concentrations in close proximity to the probable sites of action.

Despite the differences in permeability (due to the blood-brain barrier created by capillary endothelial cells), the actual concentration of LTG in the brain ECF corresponded closely to the unbound plasma concentration. This correlation was made with certain methodological considerations. Firstly, the barrier between the systemic circulation and the microdialysis probes was accounted for by the relative in vitro microdialysis probe recovery for LTG which was 26% (achieved at a flow rate of 0.5 µl/min. (which determines how effectively LTG crosses the dialysis membrane)), and secondly LTG is partly bound to plasma proteins (54%) (this remains constant over the concentration range 1-10 µg/ml) (Parsons et al., 1995). These results serve to demonstrate that steady-state LTG concentrations in the brain ECF approximate those in the unbound plasma, therefore LTG is able to distribute passively between the two fluids. Furthermore, they illustrate that the unbound plasma LTG concentration can reliably provide an accurate measure of the net effective anticonvulsant level available at the outer neuronal membrane.

As with both plasma and microdialysate LTG concentrations, whole brain LTG levels also reached steady-state at 4 days (approximately 12 µg/g), and were maintained at this stabilised concentration for the remainder of the study. However, the LTG concentration in brain was 1.5 times greater than that in plasma. This discrepancy from unity would at first perhaps suggest that equilibrium conditions governed by simple diffusion processes were not occurring. Rather, the higher levels of LTG in the brain are in part, a result of the higher lipid content of this tissue and the high lipid solubility of LTG. These findings are supported by the observations of Parsons et al. (1995) (the brain LTG concentration was 2-fold higher than that in plasma in rats following a single oral dose (2.25 - 4.0 mg/kg)), which have shown that LTG accumulates in brain by avidly binding to brain proteins and phospholipids. The binding to phospholipids depends on the partition coefficient, which is high for LTG (log P = 1.19, at pH 7.6) (Yuen, 1991).
4.4.4 Dose, plasma/brain/ECF LTG concentrations and anticonvulsant effects

Clinically, it remains unclear whether there is a direct correlation between peak plasma and brain LTG levels, neurochemical changes and an anticonvulsant effect.

The findings of this in vivo study illustrate a clear association to exist between dose and peak plasma/brain/ECF LTG concentrations and the magnitude of the inhibition of veratridine-evoked amino acid efflux. These findings are in agreement with experimental animal studies, which have reported LTG to have dose-related anticonvulsant efficacy at inhibiting hindlimb extension (Yuen, 1991).

It is interesting to note however, that the magnitude and profile of veratridine-evoked amino acid efflux achieved on day 2 of the chronic administration regimen, was not markedly different to that achieved at subsequent time-points examined up to day 21 of treatment. Since steady-state plasma/brain/ECF LTG levels take up to 4 days to achieve, the neurochemical effects on day 2 most probably reflect a residual action of the final LTG dose before commencement of microdialysis, proving to be effective since LTG has a prolonged $t_{1/2}$ of 12-15 h in rats (Parsons et al., 1995).

In summary, the findings of this in vivo study illustrate that $\text{Na}^+$ channel blockade as achieved with LTG, may have utility in the long-term treatment of epileptic seizures. This primary action may prevent excessive neuronal depolarisation and consequently, effectively limit excitotoxic excitatory amino acid (glutamate and aspartate) release through reversal of the $\text{Na}^+$-dependent amino acid transporter, thereby preserving overall ionic and excitatory : inhibitory neurotransmitter homeostasis, over a clinically effective therapeutic dose range.

At present however, clinically, LTG is being used as add-on therapy, in which the drug is tested in patients with uncontrolled seizures, and therefore, the observed effects are not truely representative of the effects of LTG itself. It is only as LTG becomes more widely employed as long-term monotherapy, that more clinical data will become available to corroborate whether these experimental observations are mimicked in humans, and furthermore the relevance of these prolonged neurochemical effects to anticonvulsant efficacy.
CHAPTER 5

A COMPARATIVE STUDY OF VOLTAGE-DEPENDENT SODIUM CHANNEL MODULATORS AND NEUROTRANSMITTER EFFLUX FROM THE RAT VENTRAL HIPPOCAMPUS IN VIVO
5.1 Introduction

Voltage-dependent Na\(^+\) channels are not only responsible for the initiation and conduction of neuronal action potentials, but can also influence presynaptic vesicular and non-vesicular neurotransmitter release (Krueger et al., 1980). During epileptic seizures however, excessive neuronal firing may underlie a detrimental influx of Na\(^+\) ions into neurones, and thereby pathologically alter neurotransmission (MacDonald and Kelly, 1995; Rogawski and Porter, 1990). Drugs that block voltage-dependent Na\(^+\) channels may therefore be capable of suppressing seizures according to the underlying seizure mechanism (Rogawski and Porter, 1990; Taylor and Meldrum, 1995).

Early experimental studies reported lamotrigine (LTG) to exhibit a pharmacological profile similar to that of the well-established, but structurally unrelated Na\(^+\) channel modulators phenytoin (PHN) and carbamazepine (CBZ) (Fig. 5(a) (Miller et al., 1986), which have extensively studied anticonvulsant mechanisms of action (reviewed by DeLerenzo, 1995; MacDonald, 1995).

In experimental seizure models, LTG, like PHN and CBZ, inhibited tonic hindlimb extension induced by either maximal electroshock or infusion of pentylenetetrazol (PTZ), but with greater potency and without increasing PTZ-induced clonus latency (Miller et al., 1986). Such effects are predictive of efficacy in partial and generalised tonic-clonic seizures. In spite of these apparent similarities, some important differences have been identified, particularly regarding effects in the electrically- and visually-evoked after-discharge tests, in which LTG alone possessed properties indicative of efficacy in absence seizures (Lamb et al., 1985; Wheatley and Miller, 1989).

Since the anticonvulsant profile of LTG resembled that of PHN and CBZ in animal seizure models, it was considered that LTG may share a similar mechanism of action at the cellular level. Both PHN and CBZ are known modulators of voltage-dependent Na\(^+\) channels, and this mechanism is considered to be responsible in part, for their anticonvulsant effects (Matsuki et al., 1984; McLean and MacDonald, 1986; Rogawski
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Fig. 5(a): Molecular structures of lamotrigine and some other conventional anticonvulsants.
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and Porter, 1990). Consequently, the site of action of LTG has been investigated in radioligand binding assays and electrophysiological experiments. These studies have shown LTG to inhibit the binding of $^3$H batrachotoxin-A-20-$\alpha$-benzoate (BTX-B) to Na$^+$ channels (Leach et al., 1991), as well as the $^3$veratrine-evoked uptake of $^{14}$C guanidine, presumably through open Na$^+$ channels (Riddall et al., 1993). PHN and CBZ also inhibit BTX-B binding, this effect is associated with reduced batrachotoxin-stimulated $^{22}$Na influx in brain synaptic terminals (Willow and Catterall, 1982).

Furthermore, PHN (Matsuki et al., 1984; Schwartz and Grigat, 1989; Ragsdale et al., 1991), CBZ (McLean and MacDonald, 1986; Schwartz and Grigat, 1989) and LTG (Cheung et al., 1992) are all capable of blocking sustained high-frequency repetitive firing (SRF) of action potentials in vertebrate cortical and spinal cord neurones in cell cultures at therapeutically effective plasma concentrations. Use- and voltage-dependent blockade of Na$^+$ channels is responsible for reducing the amplitude of Na$^+$-dependent action potentials by enhancing steady-state inactivation (i.e. reducing the rate of recovery of Na$^+$ channels from inactivation) (Lang et al., 1993; Kuo and Bean, 1994). However, whilst LTG selectively inhibits type IIa Na$^+$ channels in their slow-inactivated conformation (activated during epileptiform activity), PHN and CBZ also have an effect on the resting, closed state of the Na$^+$ channel (Xie et al., 1995).

In support of these electrophysiological findings, previous neurochemical studies have also demonstrated a Na$^+$-dependent mechanism of action for LTG on presynaptic neurotransmitter release measured from rat cortical slices in vitro (Leach et al., 1986). LTG and PHN inhibited veratrine-evoked release of the neurotransmitters aspartate, glutamate and GABA. However, LTG was slightly more effective at inhibiting the release of glutamate and aspartate than GABA (Leach et al., 1986). In contrast, K$^+$-induced neurotransmitter release (Ca$^{2+}$-dependent) was inhibited by high doses of PHN but not by LTG (Leach et al., 1986). These findings are consistent with previous observations, which have shown PHN to inhibit voltage-dependent Ca$^{2+}$ channels (Yatani et al., 1986; Twombly et al., 1988; Lang and Wang, 1991). Similarly, CBZ also blocks voltage-dependent Ca$^{2+}$ entry into synaptosomes (Ferrendelli and Daniels-
McQueen, 1982; Crowder and Bradford, 1987). In contrast, LTG at 100 μM (sufficient to block Na⁺ channels), had no significant effects on either L- or T-type Ca²⁺ channels in GH3 rat pituitary cells (Lang and Wang, 1991), although more recent evidence has demonstrated LTG to inhibit both N- and P-type Ca²⁺ currents in cortical neurones, also at concentrations effective at blocking Na⁺ conductances (Stefani et al., 1996).

These findings therefore illustrate that the characteristics of Na⁺ channel blockade produced by LTG are similar to those of PHN and CBZ (Cheung et al., 1992; Lang et al., 1993; Xie et al. 1995; Ragsdale et al., 1991), and furthermore demonstrate the inhibition of Na⁺ channels as a common site of action for structurally diverse anticonvulsants.

### 5.1.1 Useful Drug Combinations

Due to competition between sodium valproate (Na VPA) and LTG for glucuronidation, co-administration of Na VPA (200 mg) and LTG (100 mg) leads to a doubling of the t₁₂ of LTG, such that only half the normal dose of LTG is required for seizure control (Yuen et al., 1992). In addition to this pharmacokinetic interaction however, many investigators are of the general opinion that an important functional interaction between Na VPA and LTG also exists. This has been observed most conspicuously in children with atypical absences and with Lennox-Gastaut syndrome (Timmings and Richens, 1992; Panayiotopoulos et al., 1993; Pisani et al., 1993). Such children may be refractory to either drug given alone, but respond dramatically to the combination of LTG and Na VPA, suggesting that apart from the documented pharmacokinetic interaction, the two antiepileptic drugs may also be interacting pharmacodynamically, thereby summing their antiepileptic activity. Consequently, Pisani et al. (1993) have postulated that the improved efficacy observed with LTG-Na VPA co-therapy may in part, be due to complementary functional effects - LTG suppressing seizures by inhibiting excitatory neurotransmission, whilst Na VPA acting partly through a potentiation of GABAergic inhibition. A GABA-mediated mechanism is considered to be a major anticonvulsant action of Na VPA. In support of such a

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1 major metabolic route for both LTG and Na VPA.
proposal, elevations in GABA concentrations have been reported in whole brain (Godin et al., 1969), in synaptosomes (Löschner and Vetter, 1985), in hippocampal dialysates from normal (Biggs et al., 1992b), as well as convulsant (isonicotinic acid hydrazide- and MES-induced) rats, in which seizures were suppressed (Biggs et al., 1994; Rowley et al., 1995b). Interestingly however, PHN and CBZ do not reproduce this synergistic effect with Na VPA.

Furthermore, Na VPA also interacts with Na⁺ channels at concentrations found free in plasma in patients treated for epilepsy (MacDonald, 1989). As with PHN, CBZ and LTG, Na VPA is capable of limiting high-frequency repetitive firing of action potentials (SRF) in spinal cord neurones maintained in cell culture and activated by depolarising currents (MacDonald and Meldrum, 1995). However, unlike the former, Na VPA does not bind to the BTX-B binding site on Na⁺ channels (Willow and Catterall, 1982), nor does it block batrachotoxin-stimulated ²²Na-influx in brain synaptic terminals (Willow and Catterall, 1985). Therefore, it is possible that Na VPA may bind to a different site on Na⁺ channels to PHN, CBZ and LTG.

However, unlike LTG (at present), in addition to effects upon membrane conductances and synaptic function, PHN, CBZ and Na VPA also have a multitude of other reported actions (Rogawski and Porter, 1990). For example, the ability of PHN to regulate calmodulin and cyclic nucleotide second messenger systems, which are involved in numerous biochemical and physiological processes, may account for the wide diversity of its actions (reviewed by DeLerenzo, 1995). CBZ also, in addition to altering neuronal excitability, is capable of blocking NMDA receptors at therapeutically effective concentrations (Lancaster and Davies, 1992), as well as A1 adenosine receptors (Skerritt et al., 1983a, 1983b), whilst an elevation in brain GABA content and inhibition of low-threshold T-type Ca²⁺ currents, are considered to contribute to the broad-spectrum of anticonvulsant actions of Na VPA (reviewed by Fariello et al., 1995). Consequently, some of the effects produced by these drugs are anticonvulsant, whilst others are proconvulsant, but which interact to produce differing outcomes, determined by the underlying seizure mechanism.
Therefore, since excessive neuronal membrane excitability with consequent effects on Na⁺ (and Ca²⁺) ion influx, leading to a pathological alteration in neurotransmitter release, has been implicated in the pathogenesis of epilepsy (Meldrum, 1995), the objectives of the present study were to compare the neurochemical effects of the well-established Na⁺ channel modulators PHN and CBZ with those of LTG. Intracerebral microdialysis was used to investigate effects on both basal and Na⁺-dependent, veratridine-evoked amino acid and monoamine efflux from the ventral hippocampus. Furthermore, the possibility of manipulating opposite sides of the neurotransmitter balance, involving neuronal inhibition and excitation, with LTG antagonising excitation and Na VPA enhancing inhibition, have been examined.
5.2 Methods

Experimental protocols were as previously described in Section 3.2.

5.2.1 Drug preparations

LTG isethionate, Na VPA and PHN-Na were dissolved in distilled water (the latter with 1 drop 1 M NaOH), whilst CBZ was dissolved in ethanol.

Concentrations of PHN, CBZ and Na VPA were selected according to literature data which have reported effectiveness in rodent seizure paradigms (Fischer et al., 1992; Biggs et al., 1994; Rowley et al., 1995a; De Sarro et al., 1996).

Amino acid and monoamine in vitro probe recoveries

To determine whether the test compounds altered the uptake characteristics of the microdialysis probe, drugs were delivered via the probe (n=4) and in vitro amino acid and monoamine recoveries measured as described in Section 2.2.2.

5.2.2 Effects of Na⁺ channel modulators on veratridine-evoked amino acid and monoamine efflux

Since a number of tissue distribution studies in mice and rats, have shown the peak Na VPA concentration in the brain to be reached within minutes after intravenous or intraperitoneal injection (Lösch and Esenwein, 1978; Hariton et al., 1984), Na VPA was administered 30 min., rather than 90 min., prior to the second period of veratridine infusion (S2).
5.2.3 Biochemical analysis

Samples were analysed by HPLC-FD and HPLC-ED for amino acids and monoamines respectively (see Section 2.3).

5.2.4 Statistical analysis

Basal neurotransmitter time-course effects are presented in graphical form, with all values recorded as a percentage of the normalised mean basal concentrations achieved in the respective vehicle control groups. Drug effects upon veratridine-evoked neurotransmitter release are presented in tabular form, with data also expressed in percentage of control responses, with calculations based upon the comparison of S2/S1 ratios. Statistical comparisons between vehicles and drug treatments at each time-point, were made using the Mann-Whitney U test. As always p<0.05 was considered as statistically significant.
5.3 Results

Amino acid and monoamine in vitro probe recoveries

Neither PHN, CBZ, LTG, Na VPA or a combination of Na VPA and LTG altered the in vitro recoveries of amino acids or monoamines through the microdialysis probe, as compared to values determined in Table 2(a).

5.3.1 Effects of phenytoin and carbamazepine on behaviour

Following acute administration of both PHN (40 mg/kg i.p.) and CBZ (20 mg/kg i.p.), all animals adopted a flattened body posture with an abnormal spreading of the legs, and lacked exploratory activity. These effects occurred typically for up to 90 min. post-injection.

5.3.2 Comparative effects of phenytoin and carbamazepine on basal extracellular monoamine efflux

Basal concentrations of dopamine and 5-HT and their respective metabolites in ventral hippocampal dialysates were (in mean fmols ± s.e.mean/10μl dialysate): 45.80 ± 6.30 (Dopamine); 736 ± 83.20 (DOPAC); 1230 ± 357 (HVA); 87 ± 11.60 (5-HT) and 10120 ± 540 (5-HIAA) (n=33), and were calculated from the mean values of the four samples collected prior to veratridine-stimulation in all experiments.

5.3.2.1 Systemic administration of phenytoin

PHN administration enhanced dialysate dopamine efflux (by 165 ± 37 % above baseline levels), peaking 120 min. post-injection, and remaining elevated for a further 90 min., thereafter returning to baseline levels (Fig. 5(b)). However, basal DOPAC efflux was not significantly altered, unlike extracellular HVA efflux which maximally rose (by 75 ± 17 % above baseline levels) 120 min. post-administration, and correlated
with the peak dopamine effect. HVA efflux then fell in the next 60 min., before significantly rising once more in the last 60 min. of sampling (Fig. 5(b)).

PHN administration also increased basal 5-HT efflux, the maximal effect (by 214 ± 64 % above baseline levels) occurring after 60 min. Dialysate 5-HT content then declined in the next 90 min., before rising significantly again in the last 90 min. (Fig. 5(b)). In contrast, basal 5-HIAA efflux remained unaltered.

5.3.2.2 Systemic administration of carbamazepine

Following CBZ administration, basal dopamine efflux gradually increased, a maximal increase occurring 210 min. (by 51 ± 19 % above baseline levels) post-injection, before declining much more sharply (Fig. 5(b)). Concomitantly, basal DOPAC efflux was not significantly altered, unlike HVA efflux which was enhanced, exhibiting a temporal profile resembling that of dopamine efflux. Dialysate HVA content then fell slightly after 210 min., before a significant secondary rise occurred.

CBZ administration also enhanced basal 5-HT efflux (by maximally 369 ± 69 % above baseline levels) (Fig. 5(b)). The increase in dialysate 5-HT content was biphasic, rising and plateauing up to 210 min. post-administration, before increasing once more, and remaining elevated for the final 120 min. These changes were not accompanied by a significant effect upon basal 5-HIAA efflux.
Fig. 5(b): Comparative effects of acute phenytoin and carbamazepine administration on basal extracellular monoamine efflux (a-e) from the rat ventral hippocampus.

For each time-point, mean ± s.e.mean (n=5-6 animals/treatment group) is expressed as a % of the respective control value. The arrows denote the time-points at which drugs were administered i.p.; *p<0.05 represents a significant difference compared to vehicle effects (Mann-Whitney U test).

The effects of acute lamotrigine administration on basal monoamine efflux are indicated by a dotted line for comparison.
5.3.3 **Comparative effects of phenytoin and carbamazepine on veratridine-evoked monoamine efflux**

In the 30 min. sampling period proceeding veratridine (50 μM) infusion (S1), there was marked behavioural activation, consisting of increased grooming, sniffing and general exploration. Concomitant with this activity, there were significant elevations in dialysate dopamine (up to 90 % above baseline levels) and 5-HT (up to 300 % above baseline levels) efflux, together with significant reductions in the efflux of their respective metabolites (between 30-40 % below baseline levels). In comparison, in response to the second period of veratridine infusion (S2), the magnitude of the elevations in extracellular dopamine and 5-HT efflux post-PHN administration, were significantly greater than those observed proceeding S1, and most probably reflected an augmentation of the releasable dopamine and 5-HT pools, which could then be stimulated by veratridine (Table 5(a)). Veratridine-evoked HVA efflux was unchanged, whilst extracellular DOPAC levels were significantly attenuated. In contrast, stimulated 5-HIAA efflux was significantly enhanced.

Following CBZ administration, veratridine-evoked (S2) dopamine efflux was elevated. As with PHN, this effect was also most likely representative of the augmentation of basal dopamine efflux produced by CBZ. In contrast, evoked DOPAC efflux was significantly reduced, whilst the veratridine-evoked efflux of 5-HT, 5-HIAA and HVA was not significantly altered.
<table>
<thead>
<tr>
<th>MONOAMINES</th>
<th>Lamotrigine (13.4 mg/kg)</th>
<th>Phenytoin-Na (40 mg/kg)</th>
<th>Carbamazepine (20 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td>126 ± 39</td>
<td>257 ± 72**</td>
<td>174 ± 35*</td>
</tr>
<tr>
<td>DOPAC</td>
<td>108 ± 23</td>
<td>70 ± 9.0*</td>
<td>74 ± 18*</td>
</tr>
<tr>
<td>HVA</td>
<td>68 ± 26*</td>
<td>103 ± 23</td>
<td>129 ± 33</td>
</tr>
<tr>
<td>5-HT</td>
<td>113 ± 15</td>
<td>153 ± 42*</td>
<td>91 ± 31</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>89 ± 10</td>
<td>139 ± 14*</td>
<td>92 ± 9.0</td>
</tr>
</tbody>
</table>

Table 5(a): Comparative effects of the Na⁺ channel modulators lamotrigine, phenytoin and carbamazepine on veratridine-evoked monoamine efflux from the rat ventral hippocampus. Veratridine (50 μM) was infused through the microdialysis probes during samples 5 (S1) and 11 (S2), and drugs were administered i.p. after sample 7, 90 min. prior to S2. Data are means ± s.e.mean (n= 5-6 animals/group) (% of controls), calculations based on the comparisons of S2/S1 ratios; *p<0.05, **p<0.01 (Dunnett's t-test).
5.3.4 Comparative effects of phenytoin and carbamazepine on basal extracellular amino acid efflux

Basal concentrations of amino acids in ventral hippocampal dialysates were calculated to be (mean pmols ± s.e.mean/15 μl dialysate): 8.71 ± 1.23 (ASP); 43.80 ± 6.81 (GLU); 69.70 ± 8.11 (GLN); 134.8 ± 9.83 (TAU) and 2.20 ± 0.34 (GABA) (n=33), and were calculated from the mean values in the four samples collected prior to veratridine stimulation (S1).

Apart from a reduction in glutamine efflux, neither PHN nor CBZ significantly altered the basal efflux of the selected amino acids aspartate, glutamate, taurine or GABA (Fig. 5(c)).

5.3.5 Comparative effects of phenytoin and carbamazepine on veratridine-evoked amino acid efflux

Veratridine (50 μM) infusion (S1) was associated with behavioural activation as previously described (see Section 3.3.1.2.1), elevations in the efflux of aspartate (up to 250 %), glutamate (up to 125 %), taurine (up to 250 %) and GABA (up to 4000 %), and a reduction in glutamine (down to 60 %) efflux.

PHN administration significantly attenuated the veratridine-evoked (S2) efflux of aspartate, but produced no significant changes in glutamate, glutamine, taurine or GABA efflux. In comparison, CBZ had no significant effects upon veratridine-evoked aspartate, glutamate or glutamine efflux, but significantly and potently reduced the evoked efflux of the inhibitory amino acids taurine and GABA (see Table 5(b)).
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(a) 

- LTQ (13.4 mg/kg)
- PHN (40.0 mg/kg)
- CBZ (20.0 mg/kg)

(b) 

(c) 

% of control ASP efflux

% of control GLU efflux

% of control GLN efflux

Time (min.)
Comparative effects of acute phenytoin and carbamazepine administration on basal extracellular amino acid efflux (a-e) from the rat ventral hippocampus. For each time-point, mean ± s.e.mean (n=5-6 animals/treatment group) is expressed as a % of the respective control value. The arrows denote the time-points at which drugs were administered i.p.; *p<0.05 represents a significant difference compared to vehicle effects (Mann-Whitney U test).

The effects of acute lamotrigine administration on basal amino acid efflux are indicated by a dotted line for comparison.
<table>
<thead>
<tr>
<th>AMINO ACIDS</th>
<th>Lamotrigine (13.4 mg/kg)</th>
<th>Phenytoin-Na (40 mg/kg)</th>
<th>Carbamazepine (20 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>78 ± 27**</td>
<td>73 ± 29*</td>
<td>107 ± 25</td>
</tr>
<tr>
<td>Glutamate</td>
<td>27 ± 7**</td>
<td>118 ± 47</td>
<td>115 ± 24</td>
</tr>
<tr>
<td>Glutamine</td>
<td>87 ± 23</td>
<td>119 ± 35</td>
<td>147 ± 31</td>
</tr>
<tr>
<td>Taurine</td>
<td>68 ± 16*</td>
<td>87 ± 15</td>
<td>29 ± 5.0**</td>
</tr>
<tr>
<td>GABA</td>
<td>55 ± 18**</td>
<td>90 ± 23</td>
<td>36 ± 21**</td>
</tr>
</tbody>
</table>

Table 5(b): Comparative effects of the Na⁺ channel modulators lamotrigine, phenytoin and carbamazepine on veratridine-evoked amino acid efflux from the rat ventral hippocampus. Veratridine (50 μM) was infused through the microdialysis probes during samples 5 (S1) and 11 (S2), and the drugs were administered (i.p.) after sample 7, 90 min. prior to S2.

Data are means ± s.e.mean (n=5-6 animals/group) (% of controls), calculations based on the comparisons of S2/S1 ratios; *p<0.05, **p<0.01 (Dunnett's t-test).
5.3.6 Effects of drug combinations

5.3.6.1 Effects of sodium valproate on behaviour

Na VPA-treated animals adopted a hunched posture, and displayed intense "wet-dog" shake behaviour, within 1-2 min. of administration. Animals were unresponsive to external stimuli for up to 30 min. post-treatment.

5.3.6.2 Comparative effects of sodium valproate and co-administered lamotrigine on basal monoamine efflux

Na VPA (300 mg/kg i.p.) elevated extracellular dopamine (by 102 ± 65 %), DOPAC (by 348 ± 86 %) and 5-HT (by 308 ± 68 %) efflux above baseline levels (Fig. 5(d)), whereas HVA and 5-HIAA concentrations remained unchanged (Fig. 5(d)). As illustrated in Fig. 5(d), dopamine and 5-HT levels increased only transiently following Na VPA treatment (for approximately 30 min.). These effects of Na VPA upon hippocampal monoamines are consistent with the observations of Biggs \textit{et al.} (1992a).

Co-administered LTG (13.4 mg/kg i.p.) did not significantly alter the Na VPA-induced effects upon dopamine, DOPAC or HVA. However, LTG did prolong the Na VPA-induced maximal, transient rise in 5-HT efflux. The peak effect occurred 30 min. later than that observed with Na VPA alone, but remained significantly elevated for 120 min. In contrast, dialysate 5-HIAA remained unaltered.
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(a) % of control Dopamine efflux

(b) % of control DOPAC efflux

(c) % of control HVA efflux

Legend:
- □ - LTQ (13.4 mg/kg)
- ▲ - Na VPA (300 mg/kg)
- ⋄ - LTQ (13.4 mg/kg) + Na VPA (300 mg/kg)

Time (min.)
Fig. 5(d): Comparative effects of acute sodium valproate alone, and in combination with lamotrigine, on basal extracellular monoamine efflux (a-e) from the rat ventral hippocampus. For each time-point, mean ± s.e.mean (n=5-6 animals/treatment group) is expressed as a % of the respective control value. The arrows denote the time-points at which drugs were administered i.p; *p<0.05, **p<0.01 represents a significant difference compared to vehicle effects (Mann-Whitney U test).

The effects of acute lamotrigine administration on basal monoamine efflux are indicated by a dotted line for comparison.
5.3.6.3 Comparative effects of sodium valproate and co-administered lamotrigine on veratridine-evoked monoamine efflux

Na VPA did not inhibit the Na⁺-activated efflux of either dopamine or 5-HT or of their respective metabolites. In contrast, S2-evoked DOPAC and 5-HT concentrations were significantly elevated above those achieved during S1 (Table 5(c)). These effects are likely to represent the potentiating effects of Na VPA upon the basal concentrations of both DOPAC and 5-HT.

Co-administered Na VPA, normalised the inhibitory effect of LTG upon evoked HVA efflux, such that LTG-Na VPA co-therapy did not inhibit the veratridine-evoked efflux of either dopamine or 5-HT or of their metabolites. In contrast to the effects of either treatment alone, S2-evoked dopamine efflux was doubled (Table 5(c)).
<table>
<thead>
<tr>
<th>MONOAMINES</th>
<th>Lamotrigine (13.4 mg/kg)</th>
<th>Na-Valproate (300 mg/kg)</th>
<th>Lamotrigine + Na-Valproate (13.4 mg/kg) (300 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td>126 ± 39.0</td>
<td>107 ± 8.0</td>
<td>235 ± 66.0*</td>
</tr>
<tr>
<td>DOPAC</td>
<td>108 ± 23.0</td>
<td>141 ± 29*</td>
<td>148 ± 54.0</td>
</tr>
<tr>
<td>HVA</td>
<td>68.0 ± 26.0*</td>
<td>107 ± 11.0</td>
<td>102 ± 19.0</td>
</tr>
<tr>
<td>5-HT</td>
<td>113 ± 15.0</td>
<td>246 ± 49.0*</td>
<td>112 ± 14.0</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>89.0 ± 10.0</td>
<td>116 ± 11.0</td>
<td>103 ± 11.0</td>
</tr>
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</table>

Table 5(c): Comparative effects of lamotrigine and sodium valproate alone, and in combination, on veratridine-evoked monoamine efflux from the rat ventral hippocampus. Veratridine (50 μM) was infused through the microdialysis probes during samples 5 (S1) and 11 (S2). LTG was administered (i.p.) after sample 7, 90 min. prior to S2, whilst Na VPA was administered after sample 10, 30 min. prior to S2. Data are means ± s.e.mean (n=5-6 animals/group) (% of controls), calculations based on the comparisons of S2/S1 ratios; * p<0.05 (Dunnett’s t-test).
5.3.6.4 Comparative effects of sodium valproate and co-administered lamotrigine on basal amino acid efflux

Na VPA-administration elevated basal GABA efflux, whilst producing no significant changes in basal extracellular hippocampal aspartate, glutamate, glutamine or taurine concentrations (Fig. 5(e)). Peak dialysate GABA concentrations occurred approximately 60 min. post-injection. These effects are also consistent with previous \textit{in vivo} findings (Biggs et al., 1992b).

Co-administered LTG did not significantly alter the Na VPA-induced effects on basal extracellular amino acid concentrations.

5.3.6.5 Comparative effects of sodium valproate and co-administered lamotrigine on veratridine-evoked amino acid efflux

Na VPA did not inhibit veratridine-evoked amino acid efflux. S2-evoked GABA efflux was greater than that achieved during S1, and may reflect the potentiating effects of Na VPA upon GABA efflux as described in Section 5.3.6.4.

However, co-administered Na VPA did reverse the inhibitory effects of LTG upon veratridine-evoked aspartate, glutamate, taurine and GABA efflux, such that co-therapy did not significantly inhibit amino acid efflux, and moreover S2-evoked GABA efflux was significantly elevated (Table 5(d)).
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Fig. 5(e): Comparative effects of acute sodium valproate alone, and in combination with lamotrigine, on basal extracellular amino acid efflux (a-e) from the rat ventral hippocampus.

For each time-point, mean ± s.e.mean (n=5-6 animals/treatment group) is expressed as a % of the respective control value. The arrows denote the time-points at which drugs were administered i.p.; *p<0.05 represents a significant difference from vehicle effects (Mann-Whitney U test).

The effects of acute lamotrigine administration on basal amino acid efflux are indicated by a dotted line for comparison.
### Table 5(d): Comparative effects of lamotrigine and sodium valproate alone, and in combination, on veratridine-evoked amino acid efflux from the rat ventral hippocampus.

Veratridine (50 nM) was infused through the microdialysis probes during samples S1(S1) and 11 (S2), and LTG was administered (i.p.) after sample 7, 90 min. prior to S2, whilst Na VPA was administered after sample 10, 30 min. prior to S2.

Data are means ± s.e.mean (n=5-6 animals/group) (% of controls), calculations based on the comparisons of S2/S1 ratios; *p<0.05, **p<0.01 (Dunnett’s t-test).

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<td>128 ± 31.0</td>
<td>111 ± 14.0</td>
</tr>
<tr>
<td>Glutamate</td>
<td>27.0 ± 7.0 **</td>
<td>98.0 ± 17.0</td>
<td>133 ± 29.0</td>
</tr>
<tr>
<td>Glutamine</td>
<td>87.0 ± 23.0</td>
<td>122 ± 41.0 *</td>
<td>144 ± 38.0</td>
</tr>
<tr>
<td>Taurine</td>
<td>68.0 ± 16.0 *</td>
<td>112 ± 9.0</td>
<td>116 ± 31.0</td>
</tr>
<tr>
<td>GABA</td>
<td>55.0 ± 18.0 *</td>
<td>212 ± 27.0 *</td>
<td>223 ± 46.0 *</td>
</tr>
</tbody>
</table>
5.4 Discussion

The present results clearly demonstrate important quantitative and qualitative neurochemical differences to exist between LTG and the conventional antiepileptic drugs PHN, CBZ and Na VPA at anticonvulsant doses.

5.4.1 Comparative effects of phenytoin and carbamazepine on neurotransmitter efflux

Unlike LTG, PHN and CBZ administration significantly enhanced basal dopamine and 5-HT efflux from the ventral hippocampus. These data are consistent with previous findings in which similar effects have been observed both in vitro and in vivo, in both normal as well as epileptic rodents (Barros et al., 1986; Yan et al., 1992; Kaneko et al., 1993). Since Na$^+$ channel blockade for both drugs is use- and voltage-dependent (Matsuki et al., 1984; Ragsdale et al., 1991), these neurochemical effects are likely to have been produced by other actions of PHN and CBZ capable of modifying synaptic function (DeLerenzo, 1995; MacDonald, 1995). Previous studies have indeed demonstrated PHN and CBZ to be capable of regulating neurotransmitter systems (including dopamine and 5-HT) through effects on calcium and cyclic nucleotide second messenger systems (reviewed by Woodbury, 1980; Crowder and Bradford, 1987).

In contrast, of the amino acids studied, only dialysate glutamine was altered following PHN and CBZ administration, although both drugs have been shown to decrease radiolabelled excitatory amino acid release from rat brain slices (Skerritt and Johnston, 1984). PHN is also reported to decrease the concentration of glutamic acid in the brain, and increase the concentrations of glutamine and GABA in various preparations and in different species, also through effects on Ca$^{2+}$ and cyclic nucleotide second messenger systems (DeLerenzo, 1980).
5.4.1.1 Veratridine-evoked neurotransmitter efflux

The veratridine-evoked efflux of dopamine and 5-HT and their respective metabolites, was not inhibited by either LTG, PHN or CBZ (except HVA efflux following LTG administration). In contrast, amino acid efflux was differentially altered. CBZ potently reduced the efflux of the inhibitory amino acids taurine and GABA, whilst PHN modestly, but significantly, reduced evoked aspartate efflux only. LTG in comparison, was the least discriminatory, attenuating the efflux of both excitatory and inhibitory amino acids, but most potently glutamate (Chapter 3 - this thesis). In comparison, Leach et al. (1986) have previously demonstrated both LTG and PHN to inhibit the veratrine-evoked release of endogenous glutamate, aspartate and GABA in rat cortical slices. LTG was more effective at inhibiting the release of glutamate and aspartate than GABA in contrast to PHN, which inhibited glutamate and GABA release equipotently. More recently, Waldmeier et al. (1995) have reported CBZ to have a similar potency to LTG in inhibiting the veratrine-evoked release of endogenous glutamate, \(^3\)H-GABA, \(^3\)H-dopamine and \(^3\)H-5-HT also from rat brain slices. The quantitative neurochemical differences between these *in vivo* findings and the *in vitro* findings of Leach et al. (1986) and Waldmeier et al. (1995) may in part, be due to the concentrations utilised in the present study. The effects of PHN upon neurotransmitter release/ reuptake and metabolism are regulated by dose; different effects are produced at different concentrations of the drug (Woodbury, 1980), suggesting that PHN may act at different sites in regulating transmitter release and uptake, in addition to its Na\(^+\) channel modulatory activity.

Leach et al. (1986) have also demonstrated PHN to inhibit neurotransmitter release mediated by elevated K\(^+\) (Ca\(^{2+}\)-dependent) however, unlike LTG which was ineffective. PHN has been extensively reported to inhibit Ca\(^{2+}\)-ion flux across synaptic and other membranes (Sohn and Ferrendelli, 1973; Ferrendelli and Daniels-McQueen, 1982), as well as more recently to directly inhibit voltage-dependent Ca\(^{2+}\) channels (Yatani et al., 1986; Twombly et al., 1988; Lang and Wang, 1991). CBZ too, blocks voltage-dependent Ca\(^{2+}\) entry into synaptosomes (Ferrendelli and Daniels-McQueen, 1982).
Consequently, modulation of Ca\(^{2+}\)-ion fluxes across neuronal membranes is considered to be an important aspect of the actions of both PHN and CBZ. In comparison, recent evidence has demonstrated LTG to inhibit both N- and P-type Ca\(^{2+}\) currents in rat cortical neurones, at concentrations effective at inhibiting Na\(^{+}\)-conductances (Stefani et al., 1996). In clonal rat pituitary GH3 cells however, LTG at the same concentration did not inhibit high-threshold Ca\(^{2+}\) currents (L- and T-type), caused only slight inhibition of low-threshold voltage-dependent Ca\(^{2+}\) currents, reduced rapidly inactivating voltage-dependent K\(^{+}\) currents and had no significant effect on Ca\(^{2+}\)-activated K\(^{+}\) currents (Lang and Wang, 1991). These effects therefore suggest that an effect on Na\(^{+}\) channels is unlikely to underlie all the anticonvulsant effects of LTG.

Whilst both PHN and CBZ are also able to modify and inhibit voltage-dependent amino acid efflux, it would appear they may do so by mechanisms different to LTG. In support of such a proposal, Crowder and Bradford (1987) have demonstrated an inhibition of Ca\(^{2+}\) channels and Ca\(^{2+}\) sequestration within the nerve terminal to be responsible in part, for the neurochemical actions of both drugs. These investigators have shown PHN and CBZ to reduce veratrine-stimulated Ca\(^{2+}\) influx and amino acid neurotransmitter release in a dose-dependent manner in rat cortical slices. More importantly, they demonstrated that the reductions in veratrine-evoked amino acid neurotransmitter release occurred at concentrations considerably higher than those required to inhibit Ca\(^{2+}\) influx, and furthermore, greater than those required for clinical effectiveness. It is therefore possible that at concentrations of PHN and CBZ effective in rodent seizure paradigms (Fischer et al., 1992; Rowley et al., 1995a; De Sarro et al., 1996), and therefore employed in the present study, veratridine-evoked Ca\(^{2+}\) influx is more potently inhibited than Na\(^{+}\) influx. Since a large fraction of amino acids are located cytoplasmically (Nicholls, 1993), with release being Na\(^{+}\)- (Szatkowski et al., 1990; Attwell et al., 1993) and not Ca\(^{2+}\)-dependent (Nicholls, 1993; Belhage et al., 1993; Turner and Dunlap, 1995), differences in the magnitude of Na\(^{+}\) channel blockade are likely to be responsible in part, for dissimilarities in the inhibition of veratridine-evoked amino acid efflux exhibited by LTG, PHN and CBZ. At anticonvulsant doses, LTG was the most potent inhibitor of non-vesicular, Na\(^{+}\)-
dependent, cytoplasmic amino acid efflux.

In summary, although LTG, PHN and CBZ share a similar mechanism of action at the neuronal voltage-dependent Na\(^+\) channel (Lang et al., 1993), their neurochemical effects are qualitatively different regarding antiepileptic potential. Actions other than Na\(^+\) channel blockade, are likely to contribute to these effects of PHN and CBZ (see reviews by DeLerenzo, 1995 and MacDonald, 1995).

5.4.2 Drug combinations

The second part of this study extended the neurochemical comparisons between LTG and conventional antiepileptic drugs capable of Na\(^+\) channel modulation, to the neurochemical effects produced by drug interactions, in particular those between Na VPA and LTG. These effects of co-therapy are of particular interest, since a number of studies have reported improved seizure control (following failure of both drugs individually in treatment), particularly amongst children with atypical absences and with Lennox-Gastaut syndrome (Timmings and Richens, 1992; Panayiotopoulos et al., 1993; Pisani et al., 1993; Ferrie and Panayiotopoulos, 1994). These findings were recently supported in a rodent seizure paradigm, in which LTG potentiated the anti-seizure activity of Na VPA against audiogenic seizures in DBA/2 mice (De Sarro et al., 1996). Panayiotopoulos et al. (1993) have further demonstrated increased plasma LTG concentrations, due to hepatic competition for glucuronidation by co-administered Na VPA (Yuen et al., 1992), thereby doubling the half-life of LTG, as unlikely to explain the therapeutic interaction between the two drugs, since doubling the dose of LTG and removing concomitant Na VPA treatment, led to seizure relapse. Consequently, these findings led to the possibility that the beneficial effects of combining Na VPA and LTG, may be due to manipulation of opposite sides of the neurotransmitter balance, with LTG antagonising excitation and Na VPA enhancing inhibition (Pisani et al., 1993).

Co-administered LTG, did not significantly alter the effects of Na VPA upon basal
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monoamine or amino acid concentrations, with the exception of a prolongation in the transient Na VPA-induced maximal potentiation of basal 5-HT levels by LTG. A pharmacokinetic interaction seems unlikely to be responsible for producing this effect. Both clinical and animal data having demonstrated that LTG does not raise total plasma Na VPA levels (Richens, 1992; De Sarro et al., 1996). However, the possibility that LTG alters the proportion of protein binding, and thereby increases the relative free versus protein-bound ratio, may not be excluded.

Na VPA also did not inhibit monoamine or amino acid efflux from the ventral hippocampus elicited by veratridine-induced Na+ channel activation, suggesting that the magnitude by which Na+ ion influx was inhibited, may not have been sufficient to block neurotransmitter efflux at the dose administered. However, Na VPA was able to reverse the inhibition of HVA, aspartate, glutamate, taurine and GABA efflux mediated by LTG. It is unlikely that competition for Na+ channel binding sites is responsible for producing these effects. Radioligand binding studies have demonstrated that Na VPA does not bind to the BTX-B binding site on Na+ channels (Willow and Catterall, 1982) nor does it block batrachotoxin-stimulated 22Na influx in brain synaptic terminals (Willow and Catterall, 1982) unlike LTG (Leach et al., 1991). However, reversal of the LTG-evoked inhibition of GABA efflux produced by veratridine stimulation by co-administered Na VPA may in part, be due to the GABA potentiating effects of Na VPA. Though the mechanism by which Na VPA achieves this is not completely understood, an increase in GABA synthesis has been proposed as a major action. In support of this suggestion, the activity of glutamic acid decarboxylase, the enzyme responsible for GABA synthesis, was found to increase following Na VPA administration (Phillips and Fowler, 1982), and an increase in brain GABA levels was further shown to parallel the increase in enzyme activity (Nau and Löscher, 1982). Also, Na VPA has been demonstrated to inhibit the enzymes involved in GABA degradation (Godin et al., 1969; Van der Laan et al., 1979). The mechanism by which co-administered Na VPA reverses the inhibitory effects of LTG on aspartate, glutamate and taurine efflux remains unclear though, particularly since the drug has no effects on these systems alone. Furthermore, neither LTG nor Na VPA alone, or in
combination, altered amino acid or monoamine recoveries by the microdialysis probe, thereby eliminating the possibility of altered recoveries, perhaps by a physical effect of either drug, which may have influenced capture by the probe, producing a false outlook of the actual drug-mediated neurochemical effects.

These *in vivo* data have consequently demonstrated that it is not always possible to predict synergism of antiepileptic drugs based upon what is currently known about their mechanisms of action, in particular when such knowledge has come from data on seizure control. It would therefore appear that the reported increased efficacy observed with LTG-Na VPA co-therapy, may indeed in part be due to a potentiation in extracellular GABA efflux. However, an elevation in extracellular GABA was not accompanied by a concomitant reduction in excitatory amino acid efflux due to Na⁺ channel blockade as postulated by Pisani *et al.* (1993). More intriguingly, the effects of LTG-Na VPA co-therapy upon Na⁺-activated neurotransmitter efflux closely resembled the neurochemical effects produced by Na VPA monotherapy. Once again, whilst LTG does not raise total plasma Na VPA levels (Richens, 1992; De Sarro *et al.*, 1996) or increase the short half-life of Na VPA (Yuen *et al.*, 1992), an alteration in Na VPA protein binding and thereby an increase in the relative free versus protein-bound ratio produced by co-administered LTG, cannot be dismissed. Such an effect would suggest that the observed beneficial effect with Na VPA-LTG co-therapy, may be due to an elevation in the active, unbound plasma [Na VPA]. Furthermore, the failure of LTG to mediate any of the neurochemical effects which are considered to contribute to its anticonvulsant efficacy, support this suggestion. In the following chapter, an alternative mechanism of action for Na VPA has been proposed for mediating the beneficial effects described by Pisani *et al.* (1993) and other groups in children with previously refractory absence epilepsies, which may well differ from the mechanism by which an anti-seizure effect was exerted against sound-induced seizures in DBA/2 mice with Na VPA-LTG co-therapy (De Sarro *et al.*, 1996).

In conclusion, these studies have shown that whilst LTG may resemble PHN and CBZ in its use and voltage-dependent blockade of Na⁺ channels, important quantitative and
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qualitative neurochemical differences exist between LTG and these conventional Na+ channel modulators with which it has previously been closely compared. These effects may in part lead to differences in clinical efficacy according to the underlying seizure mechanism. Furthermore, the beneficial effects of combining Na VPA and LTG do not appear to involve manipulation of opposite sides of the neurotransmitter balance, thereby demonstrating that it is not always possible to predict synergism of drug combinations based upon specific, complementary mechanisms of action.
CHAPTER 6

EFFECTS OF SUB-CHRONIC (5 DAYS) LAMOTRIGINE ADMINISTRATION ON EEG SPIKE-AND-WAVE DISCHARGES AND EXTRACELLULAR AMINO ACID EFFLUX FROM THE VENTROLATERAL THALAMUS OF THE GENETIC ABSENCE EPILEPSY RAT FROM STRASBOURG (GAERS)
6.1 Introduction

Human, non-convulsive primary generalised absence epilepsies are electroclinical syndromes characterised by recurrent absence seizures, during which there are sudden interruptions of activity, vacant stares, an inability to answer questions, and no subsequent recollection of the events that occurred during the attack (Loiseau, 1992). The hallmark of these seizures is the occurrence of abnormal, bilaterally synchronous, 2.5-3.0 Hz spike-and-wave discharges (SWDs), which start and end abruptly on a normal background EEG during periods of quiet wakefulness (Marescaux et al., 1992b). At present, only a limited range of drugs are available for the management of these relatively common disorders, and so there remains a continuing need for the development of new treatments.

Generalised absence seizures arise from aberrant thalamocortical rhythms, owing to a predominance of \( \text{GABA}_\text{A} \) receptor-mediated inhibitory activity in the thalamus and/or cortex (reviewed by Snead, 1995). GABAergic dysfunction produces hyperpolarisation of neuronal membranes, thereby removing inactivation of low-threshold \( \text{Ca}^{2+} \) currents peculiar to neurones of the nucleus reticularis thalami (NRT), enabling them to generate rhythmic oscillatory activity that is necessary for the development of SWDs (Tsakiridou et al., 1995). Consequently, both \( \text{GABA}_\text{A} \) receptor antagonists and T-type \( \text{Ca}^{2+} \) channel antagonists, have been widely reported to be effective anti-absence agents (the former in only animal models at present) (Marescaux et al., 1992a; Snead, 1995, 1996a; Zhang et al., 1996a, 1996b). Conversely, since an integral part of the modulation and regulation of thalamocortical rhythmicity, is effected through glutamate-mediated recurrent excitation between thalamocortical and corticothalamic pathways, glutamatergic function via NMDA receptors also appears to be involved in the control of SWDs (Marescaux et al., 1992b). This role has been confirmed by infusing NMDA into different thalamic nuclei, which has the effect of depolarising thalamic neuronal membranes, thereby preventing the activation of low-threshold T-type \( \text{Ca}^{2+} \) currents and the onset of SWDs, in the GHB-model of absence seizures in rats (Banerjee and Snead, 1995). Furthermore, NMDA receptor antagonists have been
reported to attenuate SWD activity in a number of models of absence seizures, in
which excessive excitation is considered to be responsible for generating such seizures
(Peeters et al., 1994; Pumain et al., 1992; Snead, 1995). Other neurotransmitters (e.g.
noradrenaline, dopamine, 5-HT) are also involved in the control of SWDs, but their
roles are not as prominent as that of GABA.

Early experiments using animal seizure models, demonstrated LTG to increase clonus
latency in the PTZ-induced seizure test (Miller et al., 1986), and also to reduce the
duration of after-discharges in the electrically- and visually-evoked after-discharge tests
in rodents (Wheatley & Miller, 1989) - both these effects are considered to be
indicative of efficacy in partial seizures (Koella, 1985). Other agents effective in these
models include trimethadione, ethosuximide and sodium valproate (Marescaux et al.,
1992b), all of which are clinically employed in the management of absence seizures
(Pellock & Coulter, 1995; Ferrendelli & Holland, 1995; Bourgeois, 1995). More
recently, clinical evidence to supplement the data from the animal experimental
models, supporting the usefulness of LTG in primary generalised epilepsies, has
become available (e.g. Stewart et al., 1992; Timmings & Richens, 1992b) This includes
patients with absences resistant to previous treatment (Schlumberger et al., 1994;
Ferrie et al., 1995). In particular, Ferrie et al. (1994, 1995) and Buchanan (1995), have
demonstrated the effectiveness of LTG combined with Na VPA, this combination
being more potent than either Na VPA or LTG alone, or in combination with other
drugs.

Typically, drugs clinically employed to control generalised absence seizures (e.g.
trimethadione, ethosuximide, sodium valproate), do so by reducing the low-threshold,
T-type Ca^{2+} current in thalamic neurones, whereas agents effective against partial and
generalised tonic-clonic seizures, such as phenytoin and carbamazepine, agents with
which LTG has been closely compared, are ineffective or exacerbate (at high doses)
these seizures (Marescaux et al., 1992b). Previous in vitro and in vivo studies on the
mechanism of action of LTG have shown it to block type Ila Na^{+}-channels in a use-
and voltage-dependent manner, thereby inhibiting the veratrine-evoked release of
predominantly glutamate, but also aspartate and GABA (Leach et al., 1986; Xie et al., 1995; Chapters 3 and 4). In contrast, the effects of LTG on Ca\(^{2+}\) currents have been less consistently reported. Whilst LTG did not inhibit either L- or T-type Ca\(^{2+}\) currents in whole cell clamped pituitary cells, more recent evidence has demonstrated it to inhibit N- and P-type Ca\(^{2+}\) currents in cortical neurones at concentrations effective at blocking Na\(^{+}\) conductances (Stefani et al., 1996). Therefore, the mechanisms by which LTG exerts its anti-absence effects remain to be elucidated.

A selectively bred strain of Wistar rats, designated Genetic Absence Epilepsy Rats from Strasbourg (GAERS), has recently been validated as an experimental model of human absence epilepsy on the basis of neurophysiological, behavioural, pharmacological and genetic studies. However, the seizures differ from the human situation by the frequency at which the spontaneous SWDs occur, 6-11 Hz rather than the classical 3 Hz (Marescaux et al., 1992b). This genetic model therefore provides an experimental alternative to models in which seizures are induced by injected epileptogenic drugs, for testing the efficacies of novel drug therapies.

The aims of this study then, were to investigate the effects of sub-chronic (5 days) LTG-administration upon EEG SWD activity in the ventrolateral thalamus of GAERS. This structure was chosen since impairment of thalamic connections in the GAERS has previously been shown to result in disruption or loss of SWD activity (Vergnes and Marescaux, 1992). Furthermore, this drug regimen achieves steady-state brain and plasma LTG concentrations equivalent to those found to be clinically effective in man (see Chapter 4). Baclofen ((-) enantiomer; GABA\(_B\) receptor agonist) and Na VPA were also incorporated into the experimental design as "positive controls" and additionally, to illustrate possible synergism upon combining LTG and Na VPA. The latter part of this study used in vivo microdialysis to determine the effects of sub-chronic LTG-treatment upon extracellular amino acid efflux, implicated in the pathogenesis of absence epilepsies, also from the ventrolateral thalamus of GAERS.
6.2 Methods

GAERS (7-10 animals/group; 220-310 g) between 4-6 months old, at which age SWDs are maximally exhibited (Marescaux and Vergnes, 1995), were randomly separated into two groups, and injected intraperitoneally with either 13.4 mg/kg LTG base or its vehicle (distilled water), once daily at 09.00 h for five days. (Body weights were recorded daily). On the fourth day at 13.00 h, either microdialysis probes or bipolar EEG electrodes were implanted into the ventrolateral thalamus, under chloral hydrate anaesthesia, as previously described (see Section 2.2.3).

6.2.1 EEG monitoring

On the fifth day, all animals received their appropriate treatments at 09.00 h. They were then connected to the EEG recording/analysis system and allowed to habituate for two hours. The EEG was then continuously monitored in all animals for a two hours reference period which always commenced at 11.00 h. 2 mg/kg (-)-baclofen i.p. (in distilled water) was then injected and the EEG monitored for a further two hours, before 300 mg/kg Na VPA i.p. (in distilled water) was administered, and the EEG recorded for a final one hour. The complete preparation was earthed as previously described (see Section 2.5.2), and the animals prevented from sleeping by gentle, random sensory stimulation.

The EEG signal was amplified and filtered before being acquired, transformed and displayed, and stored for analysis on a microcomputer as previously described (see Section 2.5.3). The number, total duration and average time (s) per 30 min. period of SWDs were determined.

6.2.2 Microdialysis

Animals (10-11/group) implanted with microdialysis probes, also received their final injections at 09.00 h on the fifth day. An aCSF was infused through the microdialysis
probes for an one hour equilibration period at 0.5 µl/min. Four 30 min. basal samples were then manually collected, before the infusing aCSF was manually switched to one containing 50 µM veratridine, for a 30 min. period. The aCSF-filled pp10 polyethylene tubing was quickly disconnected from the inflow tube of the probe, and rapidly exchanged with an identical length of polyethylene tubing filled with veratridine-containing aCSF, which in turn was connected to a microsyringe mounted on the same infusion pump. At the end of the 30 min. period, the infusing solution was manually replaced with normal aCSF once more, and a further six 30 min. dialysate samples collected.

6.2.2.1 Biochemical analysis

Samples were analysed by HPLC-FD for amino acids following O-phthalaldehyde derivatisation (see Section 2.3.2).

6.2.2.2. Determination of brain, plasma and microdialysate LTG concentrations

On completion of microdialysis experiments, animals were sacrificed by stunning prior to decapitation. Blood was collected into 1 ml plasma microtainers and stored on ice until centrifugation, at 13,000 g for 10 min., in an MSE microcentaur. The plasma (supernatent) was carefully pipetted off and stored at -80°C. Brains were also rapidly removed and refrigerated at -80°C. Furthermore, 1h dialysate samples were collected from all animals prior to the commencement of microdialysate sample collection for amino acid analysis. Analysis of brain, plasma and dialysate LTG concentrations was performed by HPLC-MS (see Section 2.4).

6.2.3 Statistical Analysis

All results were expressed as a percentage of the mean basal control values. The overall effects induced by the drug treatments with respect to number, total duration and average time (s) of SWDs, and extracellular amino acid concentrations in
consecutive basal samples (30 min.) were assessed by repeated measures ANOVA followed by post-hoc multiple comparisons (Dunnett’s t-test). Additionally, statistical comparisons at equivalent time-points between the two drug treatments, in both EEG and microdialysis experiments, were performed by the Mann-Whitney U test.
6.3 Results

6.3.1 Body weights

Daily body weights steadily fell in both groups of GAERS during the five days of drug administration.

![Graph showing body weight changes over time]

Fig. 6(a): Effects of sub-chronic (5 days) LTG base- (■; 13.4 mg/kg i.p.) or vehicle- (□; distilled water) administration in GAERS. Means ± s.e.mean are shown (n=29); *p<0.05 (repeated measures ANOVA followed by Dunnett's t-test).

The mean body weight on day 5 was significantly less (5.39 %) than that on day 1 in LTG-treated GAERS, whilst there were no significant differences between columns of the vehicle-treated group.

6.3.2 Brain, Plasma and microdialysate LTG concentrations

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LTG concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brain (µg/g)</td>
</tr>
<tr>
<td>LTG</td>
<td>10.92 ± 1.11</td>
</tr>
</tbody>
</table>

Table 6(a): LTG concentrations in brain, plasma and microdialysate samples following sub-chronic (5 days) LTG-administration on daily body weights in GAERS. Means ± s.e.mean are shown (n=18 animals).
6.3.3 Behaviour

No apparent gross behavioural differences (e.g. spontaneous activity, exploration, feeding and drinking) were observed between the two treatment groups.

6.3.4 Effects of sub-chronic LTG-administration (13.4 mg/kg i.p.) on SWDs in GAERS

The characteristics of SWDs in LTG-treated animals were identical to those in vehicle-treated animals, occurring between 6-9 Hz, with similar amplitudes and bilateral symmetry (see Fig. 6(e)).

6.3.4.1 Comparison of basal EEG SWD parameters

During the first four consecutive basal 30 min. periods, there was a tendency for the SWD number/total duration to fall with time, despite efforts to prevent the animals from sleeping. This downward shift in SWD parameters occurred indiscriminately in both treatment groups, and probably represented the animals habituating to their surroundings and sensory stimulation. However, whilst this fall was not statistically significant between consecutive 30 min. periods, there were significant differences between the first and fourth 30 min. basal periods (p<0.05, repeated measures ANOVA followed by post-hoc multiple comparisons by the Dunnett’s t-test). (see Fig. 6(b)).

6.3.4.2 Effects of systemic administration of the GABA$_{	ext{A}}$ agonist (-)-baclofen

Administration of (-)-baclofen produced a comparable increase in SWD number and total duration in both LTG- and vehicle-treated rats (p<0.05, Mann-Whitney U test) (Fig. 6(b)). The aggravating effect peaked between 30-60 min. post-injection, and at the dose administered (2 mg/kg i.p.), there were no observable behavioural alterations in either group. Handling of the animals for injection, rather than an effect of drug
treatment, was probably responsible for the immediate increase in SWD number/total duration following (-)-baclofen administration, since SWD number/duration decreased in the latter half of this period. However, (-)-baclofen administration did reduce the amplitude of SWDs in both treatment groups (see Fig. 6(b)).

6.3.4.3 Effects of systemic administration of Sodium Valproate

Administration of sodium valproate (300 mg/kg i.p.) produced almost complete suppression of the (-)-baclofen aggravated SWDs in both treatment groups to a similar extent (Fig. 6(b)) (p<0.05; Mann-Whitney U test). The maximal effect occurred within 15 min. of Na VPA administration. At this dose, Na VPA produced sedation and "wet-dog" shakes. The incidence of "wet-dog" shakes was significantly greater in the vehicle-treated group (see Table 6(b)), peaking 30-45 min. post-injection.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sample 9</th>
<th>Sample 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>47.0 ± 11.70</td>
<td>11.0 ± 4.15</td>
</tr>
<tr>
<td>LTG</td>
<td>12.0 ± 3.92</td>
<td>2.0 ± 1.14</td>
</tr>
</tbody>
</table>

Table 6(b): Incidence of "wet-dog" shakes following administration of Na VPA (300 mg/kg i.p.) in GAERS sub-chronically (5 days) treated with LTG (13.4 mg/kg i.p.) or its vehicle (distilled water). Means ± s.e.mean are shown: "p<0.01 represents a significant difference from vehicle effects (Mann-Whitney U test).
Fig. 6(b): Effects of sub-chronic (5 days) LTG- (13.4 mg/kg i.p./1x daily) or vehicle- (distilled water) administration on EEG SWDs in the ventrolateral thalamus of GAERS.

Effects on (a) total No., (b) total duration (per 30 min.) and (c) mean duration (s) of SWDs. The arrows depict the time-points at which (-)-baclofen (2 mg/kg i.p.) and Na VPA (300 mg/kg i.p.) were administered respectively. Means ± s.e.mean are shown (n=8 animals/group).

*p<0.05 represents a significant difference in the total no. of SWDs between the first and fourth basal periods in both treatment groups (repeated measures ANOVA followed by post hoc multiple comparisons by the Dunnett's t-test).
Fig. 6(c): Representative EEG spectra illustrating SWDs between 6-9 Hz from the ventrolateral thalamus. Effects of (a) no further treatment and (b) (-)-baclofen (2 mg/kg i.p.) administration on EEG SWDs in a sub-chronically (5 days) LTG- (13.4 mg/kg i.p.) treated GAER.
Fig. 6(d): Representative EEG spectrum illustrating the occurrence of SWDs between 6-9 Hz from the ventrolateral thalamus.

Effect of (c) sodium valproate (300 mg/kg i.p.) administration on EEG SWDs in a subchronically (5 days) LTG-treated (13.4 mg/kg i.p.) GAER.
Fig. 6(e): Typical representative EEG SWD profiles from the ventrolateral thalamus proceeding sub-chronic (5 days) (a) vehicle- (distilled water) and (b) LTG- (13.4 mg/kg i.p.) administration. Each dotted box on the x-axis = 30 min. The arrows represent (-)-baclofen (2 mg/kg i.p.) and Na VPA (300 mg/kg i.p.) administration respectively.
Fig. 6(f): Typical representative EEG traces from the ventrolateral thalamus of a GAER proceeding sub-chronic (5 days) LTG (13.4 mg/kg i.p.) administration.
(a) No further treatment
(b) + 2 mg/kg i.p. (−)-baclofen
(c) + 300 mg/kg i.p. Na VPA.
6.3.5 Effects of sub-chronic LTG-administration on amino acid efflux in the ventrolateral thalamus of GAERS

6.3.5.1 Comparison of basal concentrations

Basal values, defined as the mean of the first four 30 min. samples, were calculated for all animals in the study, and are shown in Table 6(c).

During the four consecutive basal 30 min. periods, a steady downward shift in basal extracellular aspartate, glutamate and glutamine concentrations in vehicle-treated animals, compared to aspartate alone in LTG-treated animals, was apparent. However, this fall was not statistically significant (repeated measures ANOVA). In comparison, the extracellular GABA concentration remained stable preceding veratridine infusion.

The mean extracellular concentrations of aspartate, glutamate, glutamine and taurine were all significantly elevated in LTG-treated GAERS (p<0.05; Mann-Whitney U test; vehicle vs LTG), whilst the concentration of GABA was non-significantly altered.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Vehicle</th>
<th>LTG</th>
</tr>
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<tbody>
<tr>
<td>ASP</td>
<td>4.66 ± 1.09</td>
<td>9.34 ± 4.40</td>
</tr>
<tr>
<td>GLU</td>
<td>31.01 ± 7.56</td>
<td>38.06 ± 3.34</td>
</tr>
<tr>
<td>GLN</td>
<td>813 ± 113</td>
<td>1011 ± 67.87</td>
</tr>
<tr>
<td>TAU</td>
<td>39.0 ± 3.73</td>
<td>51.20 ± 3.23</td>
</tr>
<tr>
<td>GABA</td>
<td>1.47 ± 0.37</td>
<td>1.29 ± 0.20</td>
</tr>
</tbody>
</table>

Table 6(c): Basal amino acid concentrations in the ventrolateral thalamus of GAERS following sub-chronic (5 days) LTG- (13.4 mg/kg i.p. 1 x daily) or vehicle- (distilled water) administration.

Values are the mean ± s.e.mean (n=10-11 animals/group) of the first 4 samples; *p<0.05 represents a significant difference from control values (Mann-Whitney U test).
6.3.5.2 Effects of veratridine (50μM) on extracellular amino acid efflux in the ventrolateral thalamus of GAERS

Marked behavioural activation (e.g. increased grooming, sniffing, exploration), lasting up to 30 min., accompanied veratridine (50 μM) infusion, via the microdialysis probe, into the ventrolateral thalamus of GAERS. Veratridine infusion raised aspartate (by 22 ± 5.0 %), glutamate (by 42 ± 10 %), taurine (by 51 ± 7.0 %) and GABA (by 73 ± 9.0 %) efflux in vehicle-treated rats. In contrast, glutamine levels fell (by 94 ± 14 %) (Table 6(d)). The evoked release of aspartate and GABA peaked within 90 min. of veratridine infusion, rising sharply, and just as rapidly returning to pre-treatment values. In comparison, extracellular glutamate levels rose steadily, but this increase was sustained, peaking 120 min. proceeding the period of veratridine infusion. Similarly, the peak elevation in taurine (150 min.) and the maximum reduction in glutamine efflux were also delayed (90 min.) (see Fig. 6(g)).

However, sub-chronically LTG-treated GAERS were less behaviourally active than those of the control group in response to veratridine infusion. Concomitantly, LTG completely inhibited the veratridine-evoked efflux of aspartate and glutamate, extracellular levels were reduced maximally by 53 ± 17 % and 44 ± 16 % respectively. These inhibitory effects were long-lasting, failing to return to pre-treatment levels upon returning to the normal infusion medium. Additionally there were reductions in the magnitudes of veratridine-evoked taurine (by 26 ± 6.0 %) and significantly GABA (by 58 ± 18 %) efflux, in comparison to the elevations observed in vehicle-treated animals. Furthermore, these effects were not as sustained as those of the excitatory amino acids. In contrast, glutamine levels were reduced non-significantly less than the equivalent control value (by 88 ± 19 %), and levels in both groups did not return to baseline values for the remaining time-course of the study (see Table 6(d) and Fig. 6(g)).
## Table 6(d): Effects of veratridine (50 μM) on extracellular amino acid concentrations in the ventrolateral thalamus of GAERS following sub-chronic (5 days) LTG- (13.4 mg/kg i.p./ 1 x daily) or vehicle- (distilled water) administration.

Values are means ± s.e.mean (n=10-11 animals/group); *p<0.05 represents a significant difference from control values (Mann-Whitney U test).
Fig. 6(g): Effects of sub-chronic (5 days) LTG- (13.4 mg/kg i.p.) or vehicle- (distilled water) administration on basal and veratridine-evoked (50 μM) amino acid (A-E) efflux from the ventrolateral thalamus of GAERS. The boxes marked "V" represent the 30 min. period during which veratridine was infused through the microdialysis probe. Means ± s.e.mean (n=10-11 animals/group) are shown: *p<0.05 represents a significant difference from vehicle effects (Mann-Whitney U test).
6.4 Discussion

The results of the present study have shown sub-chronic (5 days) LTG-administration, to be ineffective at suppressing EEG SWDs from the ventrolateral thalamus, in a genetic model of absence epilepsy in the rat, despite achieving clinically effective anticonvulsant brain and plasma levels (1-3 mg/L, with some patients benefiting from concentrations up to 10 mg/L) (Leach et al., 1995). These observations are at variance with the findings of experimental animal models predictive of efficacy in generalised non-convulsive epilepsies, and also the findings of clinical studies which have demonstrated LTG to be effective in patients with previously pharmacoresistant absence seizures (see Section 6.1). These discrepant findings are discussed with relevance to the predictive value of the GAERS as an experimental model for evaluating drugs with potential anti-absence activity.

6.4.1 Body weights

Stress induced by drug administration, may in part have been responsible for producing a steady decline in daily body weights in both treatment groups. However, since weight loss was significant in LTG-treated rats, with no obvious gross behavioural dissimilarities to control animals being exhibited, differences in LTG metabolism and/or CNS handling may have led to such an effect (c.f. chronically LTG-treated normal Wistar rats in which weight loss up to 5 days was non-significant - see Chapter 4).

6.4.2 Effects of LTG on EEG SWDs from the ventrolateral thalamus in the GAERS model of absence epilepsy

Sub-chronic LTG administration did not alter EEG SWDs from the ventrolateral thalamus in GAERS. These observations are consistent with previous findings in which acutely administered LTG (6-75 mg/kg i.p.) in GAERS, failed to modify the incidence of SWDs from the frontoparietal cortex (unpublished observations,
Marescaux, 1994). A similar lack of effect with LTG at side-effect free doses (up to 50 mg/kg i.p.) has also been observed in another genetic model of absence seizures in rats, the WAG/Rij strain (Van Rijn et al., 1994).

In comparison, systemic administration of the GABA$_B$ receptor agonist (-)-baclofen, exacerbated seizure activity in GAERS, compatible with a dysfunction in GABA$_B$ receptor-mediated transmission in absence epilepsy (for review see Marescaux et al., 1992b). The aggravating effects of (-)-baclofen were not modified by LTG treatment, suggesting that GABA$_B$ receptor-mediated inhibitory mechanisms involved in the production of SWDs in GAERS, are not affected by LTG. In support of these observations, the lack of effect of LTG (up to 10$^{-4}$ M) upon $^3$H-baclofen binding to rat brain membranes further demonstrate that LTG does not interact with GABA$_B$ receptors (Leach et al., 1995).

Conversely, systemic administration of Na VPA almost completely abolished SWDs in both LTG- and vehicle-treated GAERS, agreeing with clinical observations (Stewart et al., 1992; Timmings and Richens, 1992; Ferrie et al., 1994, 1995). In particular, Ferrie et al. (1994) and more recently Buchanan (1995), have reported the effectiveness of LTG-Na VPA co-therapy, this combination being more potent than either Na VPA or LTG monotherapies. In the present study however, Na VPA administered in combination with LTG produced a comparable reduction in the incidence of SWDs to that achieved with Na VPA alone. Since the dose of Na VPA used was sufficient to almost completely suppress SWDs, a sub-maximal dose would be needed to determine whether LTG produces a synergistic effect in combination. Pisani et al. (1993) have postulated that improved efficacy with LTG-Na VPA co-therapy may in part, be due to a complementary pharmacodynamic interaction between the two drugs - LTG suppressing seizures by inhibiting excitatory neurotransmission, whilst Na VPA, acting partly through a potentiation of GABAergic inhibition. However, GABA agonists enhance seizure activity in absence models (Marescaux et al., 1992b), and therefore proposing a GABAergic mechanism to explain the action of Na VPA against absences, contradicts available experimental evidence. A more likely
explanation for the anti-absence actions of Na VPA, is its ability to block T-Type Ca\(^{2+}\) currents in thalamic neurones. Low-threshold, T-type Ca\(^{2+}\) channels, localised in the thalamus, the pontine reticular formation and the inferior olive, seem to play an important role in the genesis of SWDs. In GAERS, the nucleus reticularis thalami (NRT), which is formed of GABAergic neurones, plays a critical role in regulating SWDs (Avanzini and Marescaux, 1991; Avanzini, 1993). This role largely depends on T-type Ca\(^{2+}\) currents peculiar to NRT neurones, which are triggered via GABA\(_B\) receptor-mediated, late inhibitory post-synaptic potentials (IPSPs), enabling them to produce pacemaker rhythmical oscillatory activity, that is necessary for spike-wave generation. Na VPA produced a 16 % decrease in this current in primary afferent neurones (Kelly et al., 1991). Recently, these findings have been confirmed, with Na VPA being shown to inhibit simple thalamocortical burst activity, which resembles the SWDs of generalised absence epilepsy, in rat thalamocortical slices bathed in Mg\(^{2+}\)-free medium (Zhang et al., 1996b). Furthermore, this group have reported Na VPA to have no effect on GABA-activated Cl\(^-\) currents, observed in patch-clamp recordings of acutely isolated thalamic or cortical neurones, suggesting that the effectiveness of Na VPA against these spontaneous SWD-like rhythms, was not due to an augmentation of GABAergic inhibition, which is important in convulsive epilepsies.

6.4.3 Neurochemical effects of sub-chronic LTG administration in the GAERS model of absence epilepsy

The second part of this study focussed on some of the neurochemical abnormalities associated with generalised absence epilepsies. Whilst most neurotransmitters, including noradrenaline, dopamine and 5-HT appear to be involved in the control of SWDs in the GAERS (Marescaux et al., 1992b), there is most convincing evidence for excessive GABAergic activity underlying the generation of SWDs (Liu et al., 1991, 1992).

The findings of the microdialysis experiments, demonstrated LTG to significantly elevate the basal concentrations of both the excitatory (aspartate and glutamate) and the inhibitory amino acid taurine in the ventrolateral thalamus of the GAERS. In
contrast, comparable elevations in basal aspartate, glutamate and taurine concentrations were not observed in non-epileptic Wistar rats at 5 days, following a chronic (21 days) treatment regimen). However, important methodological constraints may have influenced these observations. In particular, since GAERS exhibit SWDs approximately only 20% of the total time (Marescaux et al., 1992a), any neurochemical changes associated with these discharges, may have been diminished by the poor time-resolution of the microdialysis technique, and further limited by the sensitivity of the detection method. Additionally, the incidence of SWDs is determined by the level of arousal (Coenen et al., 1991). Generally, fewer SWDs occur during periods of increased arousal and active behaviour and vice versa, although such behavioural differences were not apparent between LTG- and vehicle-treatments. Whilst an elevation in glutamate efflux should suppress SWDs, raised taurine efflux in contrast (which is structurally very similar to GABA, and has been reported to be increased in GAERS (Richards et al., 1995)), should be proconvulsant. However, the characteristics of SWDs were similar in both LTG- and vehicle-treatment groups, suggesting that these neurochemical changes were either of insufficient magnitude to exert an effect, or alternatively had counterbalanced one another, thereby failing to alter the overall excitatory-inhibitory neurotransmitter tone.

LTG also significantly inhibited veratridine-evoked aspartate, glutamate and GABA efflux. These effects were comparable to those exhibited in non-epileptic Wistar rats chronically-treated with LTG (see Chapter 4), suggesting that Na⁺-dependent neurotransmitter release mechanisms are normal. Furthermore, Richards et al. (1995), have recently demonstrated that the size of amino acid releasable pools in the ventrolateral thalamus of GAERS to be similar to those in non-epileptic control rats of the same strain.

Therefore in conclusion, the lack of effect exhibited by LTG in the GAERS model of absence seizures has provided conflicting evidence to human data. These discrepancies may be explained by the existence of different metabolic pathways for LTG in humans and rodents, with a human form of LTG metabolite being responsible for exerting anti-
absence effects. Alternatively, since in most cases, LTG is prescribed as add-on therapy with Na VPA, the anti-absence effects may be due to a specific pharmacodynamic interaction between the two drugs, in addition to the known pharmacokinetic interaction (i.e., an increase in the half-life of LTG caused by inhibition of its hepatic glucuronidation by Na VPA (Yuen et al., 1992)). Furthermore and perhaps more importantly, the discrepancies between animal and human data, may indicate that different molecular mechanisms underlie the thalamocortical dysfunction in GAERS and humans. However, until now, SWDs in GAERS have been a reliable predictor of drugs effective against human absences. The clinically used anti-absence drugs (e.g., ethosuximide, trimethadione, Na VPA) all suppress SWDs dose-dependently in GAERS, as well as in other experimental animal models (Marescaux et al., 1992b). It remains to be seen whether LTG is effective in other genetic and chemically-induced absence seizure models. Such information is crucial before any firm conclusions regarding the suitability of the GAERS, as an experimental model for evaluating novel, potential anti-absence drugs, can be drawn.
CHAPTER 7

CONCLUDING REMARKS
Modulation of voltage-dependent ion channels involved in action potential propagation or burst firing, is an important action of a number of well-established and newer anticonvulsants (Rogawski and Porter, 1990). Lamotrigine is a structurally unique anticonvulsant with a broad spectrum of anticonvulsant activity (reviewed by Fitton and Goa, 1995), at present considered to primarily act by stabilising neuronal membranes, via a use-dependent blockade of voltage-dependent Na⁺ channels, thereby preventing the pathologically-altered release of predominantly, the excitatory amino acid, glutamate (reviewed by Leach et al., 1995).

For a Na⁺ channel modulator to be clinically useful as an antiepileptic drug it must:

(a) not interfere with normal synaptic neurotransmission
(b) exert its effects at non-toxic plasma (unbound to plasma proteins) concentrations
(c) have a reduced side-effect profile.

These characteristics may only be determined in the whole organism, allowing for transport of the drug, and any possible interactions with other systems either by the drug itself, or compounds produced as a consequence of drug action.

However, previous studies on the mechanism of action of LTG, have generally involved the use of acute in vitro procedures, with effects being discussed in terms of direct pharmacological actions, and then correlated to plasma antiepileptic drug levels in man, to determine relevance to therapeutic efficacy. The studies presented in this thesis have therefore attempted to "bridge this gap" and complement the in vitro findings, by assessing the in vivo acute and chronic (given that LTG is administered over a prolonged period) effects of LTG, on both basal and chemically-stimulated extracellular neurotransmitter efflux, in both non-epileptic and epileptic rodents. Consequently, the findings of Chapters 3 and 4, have shown LTG to specifically and significantly inhibit the Na⁺-dependent, veratridine-evoked (rather than basal or Ca²⁺-dependent, high K⁺-evoked) release of endogenous amino acids (but not monoamines or their metabolites), primarily glutamate. But also less potently, but still significantly,
the inhibitory amino acid GABA, though this effect was not pro-convulsant, from the ventral hippocampus of non-epileptic animals, in both single- and multiple-dose studies at therapeutically relevant doses. These findings are consistent with previous in vitro data (Leach et al., 1986), and suggest that LTG may be effective at suppressing pathological excitatory amino acid-mediated neurotransmission, which may participate in abnormal discharges responsible for sustaining seizure activity, as well as in other pathological conditions associated with excessive glutamatergic neuronal activity.

Modulation of voltage-dependent Na⁺ channels is also considered to contribute to the anticonvulsant efficacy of phenytoin and carbamazepine, agents with which LTG has been closely compared and shown to resemble in anticonvulsant profile, in early experimental models (Miller et al., 1986). On the basis of these findings, in Chapter 5, the neurochemical properties of these voltage-sensitive Na⁺ channel modulators were evaluated, demonstrating important quantitative and qualitative neurochemical differences to exist, in particular the lack of effect of either PHN or CBZ upon veratridine-evoked glutamate efflux.

Also in Chapter 5, the neurochemical effects of LTG-Na VPA co-therapy were assessed, prompted by the consistent beneficial findings of a number of clinical studies which have demonstrated improved efficacy with this combination in patients with previously intractable typical absence and partial seizures (Timmings and Richens, 1992; Panayiotopoulos et al., 1993; Pisani et al., 1993). Of particular interest was the beneficial effect achieved with combining VPA and LTG, which was marked, following the failure of both drugs to produce significant seizure control individually. These findings led to the proposal that manipulation of opposite sides of the neurotransmitter balance may be responsible - LTG reducing excitation and VPA enhancing GABA-mediated inhibition (Pisani et al., 1993). Whilst this remained an exciting possibility, such complementary effects were not observed in the ventral hippocampus of non-epileptic rodents, although LTG combined with Na VPA (which was effective alone) did suppress EEG spike-and-wave discharges from the ventrolateral thalamus of GAERS, despite being ineffective alone. However, since a
maximal dose of Na VPA, sufficient to almost completely abolish SWDs was used, the possibility of an additive effect with combined LTG may have been concealed. Therefore lower dose Na VPA studies are necessary to evaluate whether a synergistic effect, not necessarily mediated by the summation of complementary antiepileptic effects produced by the drugs separately, is indeed produced with LTG-Na VPA co-therapy.

Although the mechanism(s) by which clinically refractory partial and absence seizures are improved by LTG-VPA co-therapy, could not be demonstrated in Chapters 5 and 6, these findings have illustrated that whilst it is tempting, it is not always possible to predict efficacy of drug combinations based upon complementary pharmacodynamic actions, without considering the possible influence of drug interactions.

Additionally, the ineffectiveness of sub-chronic LTG-administration in the GAERS model of absence epilepsy (Chapter 6) was unpredicted, and contradictory to the findings of animal seizure paradigms indicative of efficacy in absence seizures (reviewed by Upton, 1994), and which have been supported by observations in several preliminary clinical reports (Sander et al., 1991; Schlumberger et al., 1992; Yuen and Rafter, 1992).

Whilst the metabolism of LTG in GAERS may differ from that in man, with the human-form of LTG metabolite responsible for producing anti-absence effects, the lack of efficacy observed with LTG may refute the predictive value of the GAERS as a reliable model for screening potential drugs for anti-absence activity. However, since different molecular mechanisms may underlie such seizures in distinct genetic models of absence epilepsy (reviewed by Hosford, 1995), it may be inappropriate to rely wholly on the outcome of any single model predictive of drug activity in evaluating putative anti-absence agents. It would therefore be of interest to study whether LTG is effective in other animal models of absence seizures both genetically- (e.g. lethargic, tottering, stargazer mice) (Hosford, 1995; Noebels, 1995) and chemically- (e.g. GHB, THIP, penicillin) induced (Snead, 1992). An anti-absence mechanism of action for
LTG is made more intriguing by the findings of preclinical animal data, which have shown LTG to neither interact with GABA\(_{\text{A}}\) receptors nor to inhibit low-threshold T-type Ca\(^{2+}\) currents in rat pituitary cells, two possible targets of anti-absence therapy. However, an inhibition of T-type Ca\(^{2+}\) currents in thalamic relay neurones has yet to be demonstrated, and therefore remains an important, possible mode of action, as does inhibition of glutamatergic transmission, which is also involved in the modulation of the thalamocortical circuitry linked with the production of absence seizures.

In summary, the \textit{in vivo} findings of this thesis have demonstrated LTG to be well-tolerated, and to possess favourable pharmacological properties, which may contribute to its anticonvulsant activity. Consequently, voltage-dependent blockade of Na\(^+\) conductances may provide utility in the long-term management of certain types of epileptic seizures, by preventing excessive (but not normal) neuronal depolarisation, thereby effectively limiting excitotoxic excitatory amino acid release, and preserving overall ionic and excitatory : inhibitory neurotransmitter homeostasis, over a clinically relevant dose range. Anecdotal reports supporting particular efficacy in idiopathic generalised epilepsies have however yet to be reproduced in experimental animal models of such seizures, the points of discussion raised herein providing an important starting point for more detailed investigations to clarify how such important effects may be exerted.


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References


References


## Appendix

Table 1: International Classification of seizure types (adapted from Shorvon, 1990).

### I Partial Seizures

<table>
<thead>
<tr>
<th>A</th>
<th>Simple partial seizures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1) With motor signs</td>
</tr>
<tr>
<td></td>
<td>(2) With somatosensory or special sensory hallucinations</td>
</tr>
<tr>
<td></td>
<td>(3) With autonomic symptoms and signs</td>
</tr>
<tr>
<td></td>
<td>(4) With psychic symptoms</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B</th>
<th>Complex partial seizures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1) Simple partial onset followed by impairment of consciousness</td>
</tr>
<tr>
<td></td>
<td>(2) With impaired consciousness at onset</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C</th>
<th>Partial seizures evolving to secondary generalised seizures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1) Simple partial seizures evolving to generalised</td>
</tr>
<tr>
<td></td>
<td>(2) Complex partial seizures evolving to generalised</td>
</tr>
<tr>
<td></td>
<td>(3) Simple partial seizures evolving to complex partial seizures evolving to generalised</td>
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### II Generalised seizures

<table>
<thead>
<tr>
<th>A</th>
<th>Absence seizures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1) Atypical absence</td>
</tr>
</tbody>
</table>

| B | Myoclonic seizures |

| C | Clonic seizures |

| D | Tonic seizures |

| E | Tonic-clonic seizures |

| F | Tonic |

### III Unclassifiable epileptic seizures

1. Typically begin in the temporal lobe as simple partial leading to lapse of consciousness with blank state and orofacial automatisms (e.g. chewing). After seizure, often disorientation and anterograde memory deficits.
2. Consist of motor arrest and loss of consciousness, may be associated with blinking, myoclonic jerks /automatisms.

Typical: brief < 10 s, minimal motor manifestations, no post ictal changes. Generalised, regular 3/s or faster EEG SWDs with sudden onset/offset.

Atypical: longer, more complex motor manifestations, post ictal symptoms.

Generalised muscular jerks, high amplitude poly spike or SWDs.

Sudden loss in tone, clinically range from head drop to sudden fall.
Table II: International classification of epilepsies and epileptic syndromes and related seizure disorders (Modified from Chadwick, 1994).

1 Localisation-related (local, focal, partial) epilepsies and syndromes

1.1 Idiopathic (with age-related onset)
- Benign childhood epilepsy with centro-temporal spike
- Childhood epilepsy with occipital paroxysms
- Primary reading epilepsy

1.2 Symptomatic
- Chronic progressive epilepsia partialis continua
- Syndromes characterised by seizures with specific modes of precipitation
  - Temporal lobe epilepsies
  - Frontal lobe epilepsies
  - Parietal lobe epilepsies
  - Occipital lobe epilepsies

1.3 Cryptogenic

2 Generalised epilepsies and syndromes

2.1 Idiopathic (with age-related onset)
- Benign neonatal familial convulsions
- Benign neonatal convulsions
- Benign myoclonic epilepsy in infancy
- Childhood absence epilepsy
- Juvenile absence epilepsy
- Juvenile myoclonic epilepsy
- Epilepsy with grand mal seizures (GTCS) on awakening
- Other generalised idiopathic epilepsies
- Epilepsies with seizures precipitated by specific modes of activation

2.2 Cryptogenic or symptomatic
- West syndrome
- Lennox-Gastaut syndrome
- Epilepsy with myoclonic-astatic seizures
- Epilepsy with myoclonic absences

2.3 Symptomatic

2.3.1 Non-specific aetiology
- Early myoclonic encephalopathy
Early infantile epileptic encephalopathy with suppression burst
Other symptomatic generalised epilepsies

2.3.2 Specific syndromes
Epileptic seizures complicating other disease states

3 Epilepsies and syndromes undetermined whether focal or generalised

3.1 With both generalised and focal seizures
Neonatal seizures
Severe myoclonic epilepsy in infancy
Epilepsy with continuous spike waves during slow wave sleep
Acquired epileptic aphasia
Other undetermined epilepsies

3.2 Without unequivocal generalised or focal features

4 Special syndromes

4.1 Situation-related seizures
Febrile convulsions
Isolated seizures or isolated status epilepticus
Seizures occurring only with acute metabolic or toxic events