NEUROPHARMACOLOGICAL AND KINETIC
CORRELATES OF ANTIEPILEPTIC DRUGS
IN AN ANIMAL MODEL OF
STATUS EPILEPTICUS

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The work reported in this thesis was conducted in the:
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Dedicated to my husband, Hanneng, and my parents,
without whose love and support this work would not have been
completed
ABSTRACT

Status epilepticus (SE) is considered to be one of the most severe forms of epilepsy with significant morbidity and mortality. There is a significant need for new drug treatments and this thesis sought to investigate the neuropharmacology of three new antiepileptic drugs (fosphenytoin [FosPHT], tiagabine [TGB] and topiramate [TPM]) in an animal model of SE.

A freely moving rat model was first used to determine the temporal and concurrent kinetics of TGB and phenytoin (PHT; derived from FosPHT) in plasma, cerebrospinal fluid (CSF) and brain frontal cortex and hippocampal extracellular fluid (ECF).

TGB displayed linear kinetics in blood and CSF compartment. Time to maximal concentration (Tmax) was achieved at a mean value of 16 ± 0.3 minutes in the blood compartment, 32 ± 0.9 minutes in CSF and 41 ± 5 minutes and 34 ± 3 minutes in brain frontal cortex and hippocampal ECF compartments, respectively. The equilibration between blood and CSF compartment was reached at 30 minutes postdose, whereas the ratio of TGB concentration between ECF and serum rose over time. Distribution in the brain ECF was not brain region specific. Elimination half-life (t½) values in blood and CSF compartment were similar (50 ± 2.6 minutes and 64 ± 2.7 minutes in blood and CSF compartments respectively), but were 3 times longer in brain ECF compartment (174 ± 34 minutes and 134 ± 9 minutes in frontal cortex and hippocampus respectively).

FosPHT was rapidly metabolised to PHT and PHT displayed saturable kinetics in blood, CSF and brain ECF compartments. PHT rapidly penetrated the blood-brain barrier with a mean Tmax value of 12 ± 1 minutes in CSF, 29 ± 4
minutes and 34 ± 3 minutes in frontal cortex and hippocampus respectively. Equilibration between blood and CSF and brain ECF compartments was reached by 15-30 minutes postdose. Distribution in the brain ECF was not brain region specific. t½ values were 266 ± 64 minutes and 222 ± 70 minutes in brain frontal cortex and hippocampus respectively. Comparison of the pharmacokinetics and neuropharmacokinetics of PHT after FosPHT and PHT administration revealed comparable results.

A perforant path stimulation model of SE was used to explore the efficacy of FosPHT, TGB and TPM in suppressing seizures and protecting the brain from cell damage. Histopathological correlates (neuronal cell density), using Nissl staining, were used to identify any drug-related neuroprotective effects. Whilst TGB (at 20 mg/kg and 40 mg/kg) exhibited a dose-dependent efficacy in that SE was arrested rapidly and completely after 40 mg/kg TGB administration, FosPHT (at 100 mg/kg) and TPM (at 80 mg/kg and 160 mg/kg) were without effect in aborting SE. However, FosPHT, but not TPM, was associated with a 50% reduction of seizure severity (as assessed by Racine scale and the EEG spike frequency and amplitude). Nevertheless, none of these drugs were associated with any neuroprotection.

Using microdialysis, amino acid neurotransmitters (e.g. glutamate, γ-aminobutyric acid [GABA], and taurine) were concurrently monitored in brain ECF before and during perforant path stimulation and self-sustained SE (SSSE). GABA concentrations rose whilst glutamate and taurine concentrations fell during perforant path stimulation. In contrast, during the period post perforant path stimulation and during SSSE there was a tendency for glutamate and GABA concentrations to return to baseline level, while taurine concentrations remained suppressed.
In summary, both PHT, derived from FosPHT, and TGB have favourable kinetic features (rapid brain penetration, short Tmax at the site of drug action and slow elimination from the brain), which would be useful for the management of SE; TGB, but not FosPHT and TPM, is effective in the treatment of severe experimental refractory SE; TGB, FosPHT and TPM are without any neuroprotective properties as measured by the degree of protection from the loss of hippocampal neuronal cells in CA1, CA3 and hilus regions; the imbalance between brain excitatory and inhibitory neurotransmitters may underlie seizure occurrence and SE.
# TABLE OF CONTENTS

DEDICATION .......................................................................................................................2  
ABSTRACT ............................................................................................................................3  
TABLE OF CONTENTS ......................................................................................................5  
LIST OF TABLES ...............................................................................................................10  
LIST OF FIGURES .............................................................................................................12  
ABBREVIATIONS .............................................................................................................18  
PUBLICATIONS .................................................................................................................20  
ACKNOWLEDGEMENTS ...............................................................................................21  
DECLARATION .................................................................................................................22  

1 INTRODUCTION ......................................................................................................24  
  1.1 Overview of epilepsy and status epilepticus ...........................................24  
    1.1.1 Definition and classification ..............................................................24  
    1.1.2 Epidemiology of status epilepticus ..............................................................27  
    1.1.3 Complication and prognosis of status epilepticus ......................................29  
    1.1.4 Treatment of status epilepticus ..............................................................30  
  1.2 Pharmacokinetics during status epilepticus ............................................32  
    1.2.1 Route of drug administration and drug absorption in status epilepticus 33  
    1.2.2 Drug distribution .........................................................................................34  
    1.2.3 Hepatic metabolism .........................................................................................36  
    1.2.4 Clearance and excretion .................................................................................38  
  1.3 Drugs used in the management of status epilepticus .........................38  
    1.3.1 Diazepam ..........................................................................................................39  
    1.3.2 Lorazepam ........................................................................................................42  
    1.3.3 Phenytoin .........................................................................................................44  
    1.3.4 Propofol ............................................................................................................45  
    1.3.5 Thiopentone Sodium ......................................................................................46  
  1.4 Brain excitatory and inhibitory neurotransmitters in seizures 49  
    1.4.1 Neurochemistry of glutamate and gamma-aminobutyric acid .................49  
    1.4.2 Epileptic seizures and brain extracellular amino acids ..............................53  
  1.5 Neuronal damage from status epilepticus ..............................................54  
  1.6 Animal models of status epilepticus ...............................................................59
1.6.1 SE induced by systemic administration of chemical convulsants .......... 60
1.6.2 SE induced by focal application of chemical convulsants ...................... 60
1.6.3 Electrogenic models .................................................................................. 61
1.6.4 In vitro models .......................................................................................... 64
1.7 Drugs investigated in this thesis .................................................................. 65
1.7.1 Phenytoin .................................................................................................. 65
1.7.2 Fosphenytoin ........................................................................................... 71
1.7.3 Tiagabine .................................................................................................. 75
1.7.4 Topiramate ............................................................................................... 79
1.8 The status epilepticus model used in this thesis ........................................... 82
1.9 Aims of this thesis ....................................................................................... 83
2 MATERIALS AND METHODS ....................................................................... 85
2.1 Animals ...................................................................................................... 85
2.2 Catheters / probes construction .................................................................. 85
  2.2.1 Blood catheter construction ..................................................................... 85
  2.2.2 CSF catheter construction ........................................................................ 85
  2.2.3 Microdialysis probe construction ............................................................. 87
  2.2.4 Electrode construction .............................................................................. 88
  2.2.5 Microdialysis probe-electrode construction ............................................. 89
2.3 Surgical procedures .................................................................................... 90
  2.3.1 Blood catheter implantation ..................................................................... 90
  2.3.2 CSF catheter implantation ....................................................................... 91
  2.3.3 In vitro recovery procedure for microdialysis probes ............................... 92
  2.3.4 Microdialysis probe implantation ............................................................. 93
  2.3.5 Electrode implantation ............................................................................ 93
  2.3.6 Electrode-microdialysis probe implantation ........................................... 94
2.4 Sample collection ....................................................................................... 94
  2.4.1 Blood sample collection .......................................................................... 94
  2.4.2 CSF sample collection ............................................................................. 95
  2.4.3 Microdialysate sample collection in kinetic studies ................................. 96
  2.4.4 Microdialysate sample collection in the study of brain extracellular amino acids ......................................................................................................................... 96
2.5 Phenytoin analysis ..................................................................................... 97
  2.5.1 High performance liquid chromatography system ................................ 97
2.5.2 Reagents
2.5.3 PHT standards and quality control
2.5.4 Sample processing for PHT analysis

2.6 Tiagabine analysis
2.6.1 High performance liquid chromatography system
2.6.2 Reagents
2.6.3 TGB Standards and quality control
2.6.4 Sample processing for TGB analysis

2.7 Analysis of brain amino acids
2.7.1 High performance liquid chromatography system
2.7.2 Reagents
2.7.3 Amino acid standards and quality control
2.7.4 Sample processing for amino acid analysis

2.8 Establishment of self-sustained status epilepticus model
2.8.1 Material
2.8.2 Induction of self-sustained status epilepticus

2.9 Histology procedures
2.9.1 Preparation of 4% paraformaldehyde solution
2.9.2 Heart perfusion
2.9.3 Brain separation and fixation
2.9.4 Processing the brains
2.9.5 Sectioning and staining
2.9.6 Assessment of neuronal damage

2.10 Pharmacokinetic analysis
2.11 Statistical analysis

3 PHARMACOKINETICS AND NEUROPHARMACOKINETICS OF PHENYTOIN AFTER PHENYTOIN AND FOSPHENYTOIN ADMINISTRATION
3.3.2 Neuropharmacokinetics of phenytoin in the cerebrospinal fluid compartment ................................................................................................................125
3.3.3 Neuropharmacokinetics of phenytoin in brain extracellular fluid compartment ................................................................................................................128
3.4 Pharmacokinetics and neuropharmacokinetics of phenytoin after fosphenytoin administration ....................................................................................131
  3.4.1 Pharmacokinetics of PHT in blood compartment ...................................131
  3.4.2 Neuropharmacokinetics of phenytoin in the cerebrospinal fluid compartment ................................................................................................................133
  3.4.3 Neuropharmacokinetics of phenytoin in brain extracellular fluid compartment ................................................................................................................136
3.5 Pharmacokinetic and neuropharmacokinetic comparison of phenytoin after phenytoin and fosphenytoin administration ..............................................139
  3.5.1 Pharmacokinetic comparison of phenytoin in blood compartment after phenytoin and fosphenytoin administration ..............................................139
  3.5.2 Neuropharmacokinetic comparison of phenytoin in the cerebrospinal fluid compartment after phenytoin and fosphenytoin administration .................143
  3.5.3 Neuropharmacokinetic comparison of phenytoin in brain extracellular fluid compartment after phenytoin and fosphenytoin administration .................146
3.6 Discussion ........................................................................................................149

4 PHARMACOKINETICS AND NEUROPHARMACOKINETICS OF TIAGABINE .....................................................................................................................154
  4.1 Introduction .................................................................................................154
  4.2 Methods .......................................................................................................155
  4.3 Pharmacokinetics of tiagabine in the blood compartment .....................156
  4.4 Neuropharmacokinetics of tiagabine in the cerebrospinal fluid compartment ................................................................................................................159
  4.5 Neuropharmacokinetics of tiagabine in brain extracellular fluid compartment ..............................................................................................................163
  4.6 Discussion ...................................................................................................166

5 CHARACTERISATION OF A REFRACTORY MODEL OF STATUS EPILEPTICUS ..................................................................................................................170
  5.1 Introduction .................................................................................................170
  5.2 Methods .......................................................................................................171
5.3 Results ..............................................................................................................173
   5.3.1 Electrographic and behavioural changes..............................................173
   5.3.2 Histology ........................................................................................................175
   5.3.3 Amino acids in brain extracellular fluid..............................................180
5.4 Discussion........................................................................................................183

6 EFFICACY OF FOSPHENYTOIN, TIAGABINE AND TOPIRAMATE IN
THE TREATMENT OF EXPERIMENTAL REFRACTORY STATUS
EPILEPTICUS .................................................................................................................188
   6.1 Introduction ......................................................................................................188
   6.2 Methods ............................................................................................................189
   6.3 Efficacy of vehicle treatment in the status epilepticus .......................190
   6.4 Efficacy of fosphenytoin in the status epilepticus ................................193
   6.5 Efficacy of tiagabine in the status epilepticus ........................................196
      6.5.1 Tiagabine administration at 40 mg/kg ..............................................196
      6.5.2 Tiagabine administration at 20 mg/kg ..............................................200
   6.6 Efficacy of topiramate in the status epilepticus ..................................203
   6.7 Discussion ........................................................................................................206

7 STUDY OF THE NEUROPROTECTIVE EFFECT OF FOSPHENYTOIN,
TIAGABINE AND TOPIRAMATE IN THE MODEL OF REFRACTORY
STATUS EPILEPTICUS ........................................................................................................211
   7.1 Introduction ......................................................................................................211
   7.2 Methods ............................................................................................................213
   7.3 Histology with vehicle treatment.................................................................213
   7.4 Histology with drug treatment .....................................................................215
   7.5 Discussion ........................................................................................................217

8 GENERAL DISCUSSION ............................................................................................221
REFERENCES ..................................................................................................................230
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1.1</td>
<td>Mechanisms of chemical convulsants used to induce or exacerbate experimental SE</td>
<td>61</td>
</tr>
<tr>
<td>Table 2.1</td>
<td>PHT standards</td>
<td>101</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>Within-batch and between-batch imprecision for the measurement of PHT in serum and saline by HPLC</td>
<td>102</td>
</tr>
<tr>
<td>Table 2.3</td>
<td>TGB standards</td>
<td>105</td>
</tr>
<tr>
<td>Table 2.4</td>
<td>Within-batch and between-batch imprecision for the measurement of TGB in serum and saline by HPLC</td>
<td>107</td>
</tr>
<tr>
<td>Table 2.5</td>
<td>Amino acid standards</td>
<td>110</td>
</tr>
<tr>
<td>Table 2.6</td>
<td>Status epilepticus-motor seizure classification</td>
<td>114</td>
</tr>
<tr>
<td>Table 2.7</td>
<td>Processing protocol for rat brains</td>
<td>116</td>
</tr>
<tr>
<td>Table 3.1</td>
<td>Pharmacokinetic parameters of PHT in plasma after 30mg/kg PHT administration</td>
<td>123</td>
</tr>
<tr>
<td>Table 3.2</td>
<td>Pharmacokinetic parameters of PHT in plasma after 60mg/kg PHT administration</td>
<td>123</td>
</tr>
<tr>
<td>Table 3.3</td>
<td>Neuropharmacokinetic parameters of PHT in CSF after 30mg/kg PHT administration</td>
<td>126</td>
</tr>
<tr>
<td>Table 3.4</td>
<td>Neuropharmacokinetic parameters of PHT in CSF after 60mg/kg PHT administration</td>
<td>126</td>
</tr>
<tr>
<td>Table 3.5</td>
<td>Neuropharmacokinetic parameters of PHT in frontal cortex ECF after 60 mg/kg PHT administration</td>
<td>129</td>
</tr>
<tr>
<td>Table 3.6</td>
<td>Neuropharmacokinetic parameters of PHT in hippocampal ECF after 60 mg/kg PHT administration</td>
<td>129</td>
</tr>
<tr>
<td>Table 3.7</td>
<td>Pharmacokinetic parameters of PHT in plasma after 30mg/kg FosPHT administration</td>
<td>131</td>
</tr>
<tr>
<td>Table 3.8</td>
<td>Pharmacokinetic parameters of PHT in plasma after 60mg/kg FosPHT administration</td>
<td>132</td>
</tr>
<tr>
<td>Table 3.9</td>
<td>Neuropharmacokinetic parameters of PHT in CSF after 30 mg/kg FosPHT administration</td>
<td>134</td>
</tr>
<tr>
<td>Table 3.10</td>
<td>Neuropharmacokinetic parameters of PHT in CSF after 60 mg/kg FosPHT administration</td>
<td>135</td>
</tr>
</tbody>
</table>
Table 3.11 Pharmacokinetic parameters of PHT in frontal cortex ECF after 60 mg/kg FosPHT administration

Table 3.12 Pharmacokinetic parameters of PHT in hippocampal ECF after 60 mg/kg FosPHT administration

Table 4.1 Pharmacokinetic parameters of TGB in serum after 20 mg/kg TGB administration

Table 4.2 Pharmacokinetic parameters of TGB in serum after 40 mg/kg TGB administration

Table 4.3 TGB neuropharmacokinetic parameters in CSF after 20 mg/kg TGB administration

Table 4.4 TGB neuropharmacokinetic parameters in CSF after 40 mg/kg TGB administration

Table 4.5 Neuropharmacokinetic parameters of TGB in brain frontal cortex ECF after 40 mg/kg TGB administration

Table 4.6 Neuropharmacokinetic parameters of TGB in brain hippocampal ECF after 40 mg/kg TGB administration

Table 5.1 Neuronal density in hippocampal CA1, CA3 and hilus in control rats and saline treated SSSE rats

Table 7.1 Neuronal density in hippocampal CA1, CA3 and hilus after 100 mg/kg FosPHT, 40 mg/kg TGB, 160 mg/kg TPM and vehicle administration in SSSE rats

12
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1</td>
<td>Structural formula of diazepam</td>
<td>39</td>
</tr>
<tr>
<td>Figure 1.2</td>
<td>Structural formula of lorazepam</td>
<td>43</td>
</tr>
<tr>
<td>Figure 1.3</td>
<td>Structural formula of propofol</td>
<td>45</td>
</tr>
<tr>
<td>Figure 1.4</td>
<td>Structural formula of thiopentone sodium</td>
<td>47</td>
</tr>
<tr>
<td>Figure 1.5</td>
<td>Structural formula of PHT</td>
<td>66</td>
</tr>
<tr>
<td>Figure 1.6</td>
<td>Structural formula of FosPHT</td>
<td>72</td>
</tr>
<tr>
<td>Figure 1.7</td>
<td>Structural formula of TGB</td>
<td>76</td>
</tr>
<tr>
<td>Figure 1.8</td>
<td>Structural formula of TPM</td>
<td>79</td>
</tr>
<tr>
<td>Figure 2.1</td>
<td>Illustration of a schematic CSF catheter</td>
<td>86</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>Illustration of a microdialysis probe</td>
<td>88</td>
</tr>
<tr>
<td>Figure 2.3</td>
<td>Schematic illustration of a microdialysis probe attached to a stimulation electrode</td>
<td>89</td>
</tr>
<tr>
<td>Figure 2.4</td>
<td>PHT chromatogram</td>
<td>97</td>
</tr>
<tr>
<td>Figure 2.5</td>
<td>A typical calibration curve for PHT serum standards</td>
<td>100</td>
</tr>
<tr>
<td>Figure 2.6</td>
<td>A typical calibration curve of PHT standards for CSF samples</td>
<td>100</td>
</tr>
<tr>
<td>Figure 2.7</td>
<td>A typical calibration curve of PHT standards for microdialysate samples</td>
<td>100</td>
</tr>
<tr>
<td>Figure 2.8</td>
<td>TGB chromatogram</td>
<td>103</td>
</tr>
<tr>
<td>Figure 2.9</td>
<td>A typical calibration curve for TGB serum standards</td>
<td>106</td>
</tr>
<tr>
<td>Figure 2.10</td>
<td>A typical calibration curve of TGB standards for CSF samples</td>
<td>106</td>
</tr>
<tr>
<td>Figure 2.11</td>
<td>A typical calibration curve of TGB standards for microdialysate samples</td>
<td>106</td>
</tr>
<tr>
<td>Figure 2.12</td>
<td>Illustration of the amplifier</td>
<td>112</td>
</tr>
<tr>
<td>Figure 2.13</td>
<td>Illustration of the stimulator</td>
<td>112</td>
</tr>
<tr>
<td>Figure 2.14</td>
<td>Illustration of areas for neuron counting in the coronal section of the hippocampus from an unstimulated rat</td>
<td>118</td>
</tr>
<tr>
<td>Figure 3.1</td>
<td>Plasma PHT concentration versus time profiles after 30 mg/kg and 60 mg/kg PHT administration</td>
<td>124</td>
</tr>
<tr>
<td>Figure 3.2</td>
<td>Plasma free / total PHT concentration ratios after 30</td>
<td>124</td>
</tr>
</tbody>
</table>
Figure 3.18  PHT concentration versus time profiles in CSF after 30 mg/kg and 60 mg/kg FosPHT and PHT administration

Figure 3.19  Dose-adjusted PHT Cmax in CSF

Figure 3.20  Dose-adjusted PHT AUC in CSF

Figure 3.21  PHT t½ in CSF

Figure 3.22  CSF / plasma PHT concentration ratios after 30 mg/kg PHT and FosPHT administration

Figure 3.23  CSF / plasma PHT concentration ratios after 60 mg/kg PHT and FosPHT administration

Figure 3.24  PHT concentration in frontal cortex ECF versus time profiles after 60 mg/kg FosPHT and PHT administration.

Figure 3.25  PHT concentration in hippocampal ECF versus time profiles after 60 mg/kg FosPHT and PHT administration.

Figure 3.26  Frontal cortex ECF / plasma PHT concentration ratios after 60 mg/kg PHT and FosPHT administration

Figure 3.27  Hippocampal ECF / plasma PHT concentration ratios after 60 mg/kg PHT and FosPHT administration

Figure 4.1  TGB serum concentration versus time profiles after 20 mg/kg and 40 mg/kg TGB administration

Figure 4.2  Dose-adjusted TGB serum Cmax values

Figure 4.3  Dose-adjusted TGB serum AUC values

Figure 4.4  TGB concentration versus time profiles in CSF after 20 mg/kg and 40 mg/kg TGB administration

Figure 4.5  Dose-adjusted TGB Cmax values in CSF

Figure 4.6  Dose-adjusted TGB AUC values in CSF

Figure 4.7  CSF / serum TGB concentration ratios after TGB administration at 20 mg/kg and 40 mg/kg

Figure 4.8  TGB concentration versus time profiles in brain frontal cortex and hippocampal ECF after 40 mg/kg TGB administration

Figure 4.9  ECF / serum TGB concentration ratios in brain frontal
cortex and hippocampus after 40 mg/kg TGB administration

Figure 5.1 Dentate granule cell field potential 173
Figure 5.2 Multiple population spikes during stimulation 174
Figure 5.3 Percentage change of spike frequency and amplitude from baseline value in saline treated SSSE rats at different time window after the end of perforant path stimulation 175
Figure 5.4 EEG of a control rat and a saline treated SSSE rat at different stage of seizure development 176
Figure 5.5 Histology of hippocampus and close up of CA1 from an unstimulated control rat and a saline treated SSSE rat 178
Figure 5.6 Neuronal density on the left and right sides of hippocampus in the saline treated SSSE group 179
Figure 5.7 Neuronal density in three regions of the hippocampus in the unstimulated control group and the saline treated SSSE group 179
Figure 5.8 Percentage change of glutamate, GABA and taurine concentrations in relation to baseline in the hippocampal ECF during and after perforant path stimulation 181
Figure 5.9 Percentage change of glycine, alanine and glutamine concentrations in relation to baseline in the hippocampal ECF during and after perforant path stimulation 182

Figure 6.1 The EEG of a rat in vehicle treated group 191
Figure 6.2 Percentage change of spike amplitude in relation to baseline value in vehicle and saline treated animals during the 3-hour observation period 192
Figure 6.3 Percentage change of spike frequency in relation to baseline value in vehicle and saline treated animals during the 3-hour observation period 192
Figure 6.4 The EEG of a rat in 100 mg/kg FosPHT treated group 194
Figure 6.5 Change in seizure severity after 100 mg/kg FosPHT administration 195
Figure 6.6 Percentage change in spike amplitude in relation to baseline value in rats after 100 mg/kg FosPHT administration

Figure 6.7 Percentage change in spike frequency in relation to baseline value in rats after 100 mg/kg FosPHT administration

Figure 6.8 Change in seizure severity after 20 mg/kg and 40 mg/kg TGB administration

Figure 6.9 The EEG of a rat in 40 mg/kg TGB treated group

Figure 6.10 Percentage change in spike amplitude in relation to baseline value in rats after 40 mg/kg TGB administration

Figure 6.11 Percentage change in spike frequency in relation to baseline value in rats after 40 mg/kg TGB administration

Figure 6.12 The EEG of a rat in 20 mg/kg TGB treated group

Figure 6.13 Percentage change in spike amplitude in relation to baseline value in rats after 20 mg/kg TGB administration

Figure 6.14 Percentage change in spike frequency in relation to baseline value in rats after 20 mg/kg TGB administration

Figure 6.15 The EEG of a rat in 80 mg/kg TPM treated group

Figure 6.16 Change in seizure severity after 80 mg/kg TPM administration

Figure 6.17 Percentage change in spike amplitude in relation to baseline value in rats after 80 mg/kg TPM administration

Figure 6.18 Percentage changes in spike frequency in relation to baseline value in rats after 80 mg/kg TPM administration

Figure 7.1 Hippocampal histology and close up of CA1 from animals in SSSE treated with vehicle, FosPHT 100
mg/kg, TGB 40 mg/kg and TPM 160 mg/kg

Figure 7.2 Neuronal density in hippocampal CA1, CA3 and hilus in control, saline treated and vehicle treated groups

Figure 7.3 Neuronal density in hippocampal CA1, CA3 and hilus in FosPHT, TGB, TPM treated groups, compared to vehicle treated group
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AED</td>
<td>antiepileptic drug</td>
</tr>
<tr>
<td>AD</td>
<td>afterdischarge</td>
</tr>
<tr>
<td>AMPA</td>
<td>alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the concentration versus time curve</td>
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<tr>
<td>BBB</td>
<td>blood-brain barrier</td>
</tr>
<tr>
<td>BCB</td>
<td>blood-CSF barrier</td>
</tr>
<tr>
<td>CBZ</td>
<td>carbamazepine</td>
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<tr>
<td>Cmax</td>
<td>maximal concentration</td>
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<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CPP</td>
<td>3(2-carboxypiperazine-4-yl)propyl-1-phosphonic acid</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>CYP</td>
<td>cytochrome P450</td>
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<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DZP</td>
<td>diazepam</td>
</tr>
<tr>
<td>ECF</td>
<td>extracellular fluid</td>
</tr>
<tr>
<td>EEG</td>
<td>electroencephalograph</td>
</tr>
<tr>
<td>FosPHT</td>
<td>fosphenytoin</td>
</tr>
<tr>
<td>GABA</td>
<td>gamma-aminobutyric acid</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>Ke</td>
<td>elimination rate constant</td>
</tr>
<tr>
<td>LDH</td>
<td>lactate dehydrogenase</td>
</tr>
<tr>
<td>ILAE</td>
<td>International League Against Epilepsy</td>
</tr>
<tr>
<td>IM</td>
<td>intramuscular</td>
</tr>
<tr>
<td>IP</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>IV</td>
<td>intravenous</td>
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<tr>
<td>LZP</td>
<td>Lorazepam</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
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<tr>
<td>LTG</td>
<td>lamotrigine</td>
</tr>
<tr>
<td>MES</td>
<td>maximal electroshock test</td>
</tr>
<tr>
<td>min</td>
<td>minute</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>PB</td>
<td>phenobarbitone</td>
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<tr>
<td>PE</td>
<td>PHT equivalent</td>
</tr>
<tr>
<td>PHT</td>
<td>phenytoin</td>
</tr>
<tr>
<td>p-HPPH</td>
<td>5-(4-hydroxyphenyl)-5-phenylhydantoin</td>
</tr>
<tr>
<td>PKa</td>
<td>absorption rate constant</td>
</tr>
<tr>
<td>PPR-I</td>
<td>precipitating reagent for PHT analysis</td>
</tr>
<tr>
<td>PPR-II</td>
<td>precipitating reagent for TGB analysis</td>
</tr>
<tr>
<td>SE</td>
<td>status epilepticus</td>
</tr>
<tr>
<td>SSSE</td>
<td>self-sustained status epilepticus</td>
</tr>
<tr>
<td>TGB</td>
<td>tiagabine</td>
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<tr>
<td>TLE</td>
<td>temporal lobe epilepsy</td>
</tr>
<tr>
<td>t½</td>
<td>elimination half-life</td>
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<tr>
<td>Tmax</td>
<td>time to maximal concentration</td>
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<tr>
<td>TPM</td>
<td>topiramate</td>
</tr>
<tr>
<td>VPA</td>
<td>valproic acid</td>
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PUBLICATIONS


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DECLARATION

All of the work described in this thesis was undertaken by the author, with the exception of the measurement of TGB concentrations, which was undertaken by Dr N Ratnaraj, and the measurement of brain extracellular amino acid concentrations, which was undertaken by Dr G Sills.
1 INTRODUCTION

1.1 Overview of epilepsy and status epilepticus

1.1.1 Definition and classification

The word epilepsy is derived from the Greek verb “epilamvanein” (to be seized, to be taken hold of or to be attacked), that is derived from the even older notion that all diseases represented attacks by the gods or evil spirits, usually as punishment. Because seizures were the most vivid example of demonic possession, epilepsy was considered to be “the sacred disease”. Indeed, the battle between prejudice and acceptance, ignorance and knowledge, myth and science has been long and difficult. Great attribution should be paid to Hippocrates in about 400 BC who defined epilepsy as a disease of the brain that must be treated by diet and drugs, not religious incantations (Page et al., 1967).

Modern concepts of epilepsy originated from the work of mid-19th century physicians and scientists, the most important among them being John Hughlings Jackson (Taylor, 1993). At a time when epilepsy denoted disorders manifested by generalised convulsions, which were believed to arise from disturbances in the medulla oblongata, Hughlings Jackson established the important concept that there were different categories of seizures, each with its own physiology and semiology. His explanation of “dreamy states” and “uncinate group of fits” as partial seizures originating from discrete areas within the cerebral cortex comes close to present-day views of complex partial seizures not only identified the responsible locus within the brain but allowed him to draw differences that have forever changed our concepts of cortical motor representation and cerebral control of voluntary movement.
In contemporary epileptology, epilepsy is not a specific disease, or even a single syndrome, but rather a broad category of symptom complexes arising from any number of disordered brain functions that themselves maybe secondary to a variety of pathologic processes. Epilepsy is defined as a condition characterised by recurrent (two or more) epileptic seizures, unprovoked by any immediately identified cause. Epileptic seizure is a clinical manifestation presumed to result from an abnormal and excessive discharge of a set of neurons in the brain (Shorvon, 1994). The clinical manifestation consists of sudden and transitory abnormal phenomena, which may include alterations of consciousness, motor, sensory, autonomic or psychic events, perceived by the patient or an observer. A variety of conditions or epilepsies have been categorised and defined not only by the types of seizures they manifest but also by other associated clinical features. Specific epileptic syndromes have been identified by their characteristic seizure types, pattern of seizure recurrence, age of onset, associated neurological and other clinical signs, electroencephalographic findings, presence or absence of familial occurrence, and prognosis.

Epilepsy and epileptic syndromes are broadly divided into idiopathic and symptomatic disorders. Idiopathic epilepsies are generally benign in the sense that they are not associated with brain lesions, neurological abnormalities other than seizures, or mental impairment, and that they tend to be self-limited or respond readily to antiepileptic drugs (AEDs). Symptomatic epilepsies are those in which seizures are consequence of an identifiable lesion or other specific aetiology. When epilepsies are presumably symptomatic, but currently of unknown specific aetiology, they are defined as cryptogenic.
Attempts at classification of seizures and epilepsy as observed natural phenomenon have formed part of medical history from the beginning. The classification evolved over the years aiming to reflect the advancement of knowledge of epilepsy. The latest version of the classification of seizures, epilepsies and epileptic syndromes was proposed in 1981 and 1989 respectively by the International League Against Epilepsy (ILAE). Over recent years, the advancement of neurodiagnostic technology and rapid development in molecular genetics has greatly improved our understanding of epileptic seizures and epileptic disorders. It has become clear that more is needed for specific applications of growing importance, such as presurgical evaluation, clinical pharmacology trials, and epidemiological studies. Therefore a new version of ILAE classification and terminology is underway (Engel, 1998).

Status epilepticus (SE) is the maximal expression of epilepsy. The necessity of differentiating SE from other seizures is not solely clinical, but relates most importantly to the high morbidity and mortality characterised by this condition (Walker et al., 1995b). SE is a condition in which epileptic activity persists for 30 minutes or more, causing a wide spectrum of clinical symptoms, and with a highly variable pathophysiological, anatomical and aetiological basis (Shorvon, 1994). SE is not simply rapid repetition of seizures but a condition (or group of conditions) with distinctive pathophysiological features. This definition avoids the term epileptic seizure and thus the difficulties of definition and classification of seizures. It allows the incorporation of conditions that do not conform to a ‘seizure type’ and conditions where there is continuous electrographic ictal activity causing persisting symptoms but no discrete seizures, thus including many types of nonconvulsive SE. Excluded, however are cases exhibiting persistent electrographic activity in the total absence of
clinical symptoms, as might occur for instance with inter-ictal spiking. A minimum limit of 30 minutes is set to render the definition clinically useful. The diversity of clinical features of SE is also recognised, as is the highly variable pathophysiological and aetiological basis.

1.1.2 Epidemiology of status epilepticus

Despite the frequency and effects of SE, there have been few good epidemiological studies of SE, and only recently have there been population-based studies. Because of the differences in ascertainment methods of SE and the different population base used in the study, data from different studies differ significantly. Taking into account the factor that many cases may have been missed or patients died before reaching hospital, the figures can only be broad estimates.

In a prospective population-based study in Richmond, Virginia, USA (Delorenzo et al., 1996), the overall incidence was estimated as 41 - 61 per 100 000 person-year with a mortality of 9 - 17 per 100 000 person-year. Overall, 13.3% had recurrent attacks, 58% had no previous history of SE and the mortality was 22%. The elderly had the highest incidence of attacks with the greatest mortality (38%) and the least history of prior epilepsy (30%). Although the prognosis of SE was related to aetiology, the prognosis of certain conditions, such as stroke appeared to be very much worse if they were associated with SE than if they were not.

Three further population based studies of SE in Rochester, Minnesota, (Hauser et al., 1993; Logroscino et al., 1997; Hesdorffer et al., 1998) reported age-adjusted incidence of SE to be 16 - 21 per 100 000 person-year (Hesdorffer et al., 1998). This is substantially lower than that in Richmond study. This may in part be due to the different ascertainment methods used and mild forms of SE being missed.
from both the patient side and the misdiagnosis in the hospital. A further confounding factor is the population base that differs extensively in race and class between the two areas (Richmond has a much higher proportion of non-whites). The incidence peaked during first year of life and then again after age 65 years. SE tended to be of longer duration at these age groups (Hesdorffer et al., 1998). SE was more common in males than in females, which can partly be explained by the increased incidence of certain aetiology in the male population, but may also relate to the effect of hormonal influences on seizure termination (Hesdorffer et al., 1998).

In the Rochester study, SE was classified as febrile in 8%, acute symptomatic (within one week of a precipitating insult) in 50% and unprovoked in 42% (two-thirds of these had a progressive or remote symptomatic cause). Over half of the SE occurred in the absence of epilepsy (Hesdorffer et al., 1998). The main acute symptomatic causes identified in the Richmond study were: infections with fever in children, low AED concentrations, cerebrovascular accidents, hypoxia, metabolic disturbance and alcohol in adults (Delorenzo et al., 1996).

AED withdrawal in those with epilepsy has frequently been cited as a major cause of SE. It was even proposed that SE was a condition that resulted from the advent of powerful AEDs, and the consequent risk of drug withdrawal (Hunter 1959). However some evidence suggested this was not the case (Barry et al., 1994; Maytal et al., 1996).

SE is common in children with symptomatic epilepsy. Using logistic regression analysis in a case control study of 44 children with symptomatic epilepsy who had an episode of SE (Novak et al., 1997), four factors emerged as significant predictors of SE: focal background abnormalities on electroencephalograph (EEG),
history of partial seizures with secondary generalisation, generalised abnormalities on neuroimaging and first seizure as SE.

**1.1.3 Complication and prognosis of status epilepticus**

Two of the major consequences of SE are unprovoked seizures and death. The frequency of these outcomes was determined in two studies from Rochester, USA (Logroscino et al., 1997; Hesdoffer et al., 1998). Acute symptomatic seizures are rarely associated with the later development of unprovoked seizures. Hesdorffer (1998) looked at the influence of acute symptomatic SE on the later development of unprovoked seizures over a 10-year period. Of those with acute symptomatic seizures with SE, 43% had a subsequent unprovoked seizure compared with 13% of those with acute symptomatic seizures without SE. Controlling for age, sex and aetiology, SE increased the risk of unprovoked seizures after an acute symptomatic seizure 2.9 fold. The highest relative risk was for acute symptomatic seizures with SE and an underlying structural cause. Whether the influence of SE is just as a marker of a more severe insult or whether SE itself increases the probability of later developing epilepsy is unknown.

The high mortality of SE has been further confirmed in the other study from Rochester (Logroscino et al., 1997). In this study, the overall mortality was 21%. Most of these deaths (89%) occurred in those with acute symptomatic aetiology, especially anoxic encephalopathy or cerebrovascular disease. When analysed for other factors, only age (> 65 years) and sex (male) contributed significantly to the risk of death. This appeared to be independent of aetiology. In the overall analysis, however, length of SE was not an independent predictor of
mortality. Whether it is the underlying aetiology itself or the SE that has a major influence on mortality cannot be determined from this study.

1.1.4 Treatment of status epilepticus

Initial stages

The initial stages of SE are treated with a combination of a benzodiazepine (usually diazepam [DZP] or lorazepam [LZP]), phenytoin (PHT) or phenobarbitone (PB) administered intravenously (Walker et al., 1995b). This should be followed by regular AED treatment in order to maintain therapeutic AED concentrations.

Benzodiazepines are often the first treatment. DZP, however, has a short redistribution half-life (< 1 hour) and a large volume of distribution of 102 L/kg (Schmidt, 1995). This results in a rapid fall in serum concentration and a concomitant fall in brain concentration, leading to recurrence of seizure activity (Greenblatt et al., 1990; Schmidt, 1995; Walker et al., 1998). For this reason, repeat intravenous (IV) boluses or a continuous infusion of DZP are commonly used. Accumulation occurs, and with DZP's long elimination half-life (t½) of 30 h, there is a risk of prolongation of action. This could lead to sudden hypotension, and respiratory and circulatory collapse.

In recent years LZP has been identified as a better option in the initial treatment of SE in that it has a prolonged pharmacological action, which makes continuous or frequent administration unnecessary, and a high degree of freedom from serious side effects involving either the respiratory and cardiovascular system (Homan et al., 1983; Leppik et al., 1983).
Two recent advances in the initial treatment of SE have been the use of intranasal medication, and the launch of fosphenytoin (FosPHT), a PHT pro-drug. A complication that occurs in Casualty Departments is difficulty or delay in getting IV access. In these cases, rather than delay treatment, an alternative route for drug administration needs to be used.

Midazolam has the advantage over other benzodiazepines in that it is water soluble at a suitable pH; at physiological pH it becomes highly lipophilic, permitting rapid transfer across the blood-brain barrier (BBB) (Pieri, 1983). It has previously been used both as an IV and as an intramuscular (IM) treatment for acute seizures (Nordt et al., 1997). Midazolam can also be administered intranasally. A dose of 0.2 mg/kg intranasally results in a maximum serum concentration in 12 min with a bioavailability of 55% (Rey et al., 1991).

Parental PHT is relatively insoluble in water and has a high pH. It consequently has a number of side effects related to its physiochemical properties (Browne, 1997). It may crystallise and precipitate in solution; it may cause thrombophlebitis (particularly with extravasation); its vehicle, propylene glycol, can cause hypotension; and it is poorly and erratically absorbed after IM injection (Browne, 1997). In order to overcome these problems, FosPHT (3-phosphoryloxymethyl PHT disodium), a water-soluble PHT pro-drug, was developed. Although it is more expensive than PHT, these costs may be balanced by less phlebitis, less hypotension, ease of administration and greater tolerability (Browne, 1997).
Later stages

After failure of initial treatment, early referral to an intensive care unit is mandatory because the late stages of convulsive SE are associated with hyperthermia, acidosis, cardiac arrhythmia, hypotension, pulmonary oedema, rhabdomyolysis, acute renal failure, hepatic compromise and disseminated intravascular coagulation. Intubation and mechanical ventilation not only aid the treatment of many of these complications, but also the use of general anaesthetic agents, which is an effective means of halting both motor and electrographic seizure activity.

The choice of anaesthesia in SE remains a matter of debate. The barbiturate anaesthetics (e.g. thiopentone, pentobarbitone) have the advantage of long experience in the treatment of SE, putative cerebroprotective action and potent antiepileptic activity (Opitz et al., 1983; Brown et al., 1967). The main disadvantage, however, is the saturable pharmacokinetics of thiopentone, resulting in protracted recovery time in prolonged therapy. They also commonly result in hypotension, and concomitant inotropes are often required.

Both propofol and midazolam have been proposed as alternatives to barbiturate anaesthesia. Propofol has very suitable kinetics (high lipid solubility therefore rapid entry into brain and relatively longer t½ than DZP), and comparable efficacy to that of midazolam and barbiturate anaesthetics in terminating refractory SE (Prasda et al., 2001; Stecker et al., 1998).

1.2 Pharmacokinetics during status epilepticus

Knowledge of drug pharmacokinetics is essential in achieving successful treatment of SE. Indeed during SE, the pathophysiological status of patients alters
significantly, which increases the complexity of the treatment. Rational treatment regimens based on pharmacokinetic principles can greatly improve the outcome of SE.

1.2.1 Route of drug administration and drug absorption in status epilepticus

In SE, the first priority is to deliver adequate quantities of drugs as fast as possible to the epileptic brain tissue. IV short-term infusion, bolus injection or sometimes continuous infusion is the route of choice in most instances. The advantages of IV administration are obvious: bioavailability is 100%, time to maximal concentration (Tmax) is very short, and there are no gastrointestinal factors affecting absorption (Shorvon, 1994). Caution though, is vital. Many drugs require dilution, but if diluted in unsuitable concentrations or inappropriate solvents may precipitate out of solution (e.g. a common problem with PHT). Co-medication may also cause precipitation (e.g. of PHT if the pH of the solution is low). For these reasons, the combined administration of DZP and PHT requires two separate IV lines. Drugs such as paraldehyde react rapidly with plastic giving sets or solvent bags, and DZP and thiopentone, amongst others, do so on prolonged contact. The IV injection of decomposed or chemically altered drugs may be dangerous, a particular problem with paraldehyde. The rate of injection is critical; too fast a bolus injection may result in massive cerebral uptake on first pass, causing respiratory arrest (e.g. DZP) and asystole (e.g. PHT) (Shorvon, 1994). It is essential, when administering any drug via IV route, to monitor clinical and cardiorespiratory function continuously and be prepared to modify the rate and the dose given.
Rectal administration is a safe and convenient method, without the local or systemic risks of IV administration. The instillation of a solution, but not the use of wax suppositories, of DZP, midazolam and paraldehyde produces reliable and rapid absorption of the drug.

A few drugs can be given intramuscularly, notably paraldehyde and midazolam. Most, however, cannot. Neither DZP nor PHT should be given via the IM route because absorption is slow and unreliable (PHT may in fact crystallise in muscle). No drug is absorbed fast enough by the oral route to satisfy the requirement in SE. Nor should any AEDs be given intra-arterially in SE because of the high risk of arterial spasm, necrosis, thrombosis and embolism (Shorvon, 1994).

1.2.2 Drug distribution

The most important properties determining distribution are lipid solubility and to a lesser extent, the degree of ionisation (Shorvon, 1994). Cerebral blood vessels are lined with a unique arrangement of endothelial cells (the BBB), which allow less molecular transfer than elsewhere in the body, and pose a barrier to egress into brain tissue of blood-borne substances. Only small molecules or ions, molecules for which there are specific transfer systems, or highly lipid soluble molecules cross the barrier readily (Cornford et al., 1986).

In SE, the lipid solubility properties of a drug usually determine the rate of entry into the brain and thus the value of a drug (Cornford et al., 1986). The higher the lipid solubility, the faster will be the time to peak brain concentrations after IV injection. Strongly ionised drugs also will not enter cerebral tissue easily. Ionisation can be predicted from the acidity or basicity of the compound, its absorption rate constant (pKa) and the ambient pH. In SE, pH can fall progressively, increasing the
ionisation of basic drugs (e.g. DZP) and decreasing the ionisation of acidic drugs (e.g. thiopentone) (Shorvon, 1994). In contrast status-induced changes in pH will have little effect on amphoteric drugs, such as clonazepam and LZP.

None of the drugs given in SE is distributed equally and instantly throughout the body (i.e. the body acting as a single compartment) (Shorvon, 1994). This is particularly true of lipid-soluble drugs, which are preferentially concentrated in lipid tissue. For most drugs given in SE, the two-compartment or three-compartment models reasonably accurately describe IV distribution data. The central compartment comprises blood and the extracellular fluid (ECF) of the highly perfused organs (e.g. brain, liver and kidney); the peripheral compartments comprise the less perfused tissues (e.g. muscle and fat). For most lipid soluble drugs (with linear kinetics) after an IV bolus, blood concentrations fall biphasically over time. The two-compartment model provides an explanation for this phenomenon. The first phase largely represents distribution, and the second elimination. Distribution accounts for the rapid fall in blood (and brain) concentration. In the elimination phase, the removal of drug from the blood is dependent on metabolism. The two-compartment-model theory has the consequences of great practical importance in SE: after a first IV injection of lipid-soluble drugs, concentrations are initially very high and then fall rapidly. Thus the initial drug effects are transient; after repeated doses, the peripheral tissues contain a larger proportion of the cumulative dose and constitute a reservoir of drug. Furthermore, the central compartment (and thus the brain) concentrations are sustained by drugs returning from the peripheral reservoir. With administration of further drug, the rapid distribution from blood to peripheral compartment will thus not readily occur. At this stage, further IV injections will cause blood concentrations to peak as before, but there will be no rapid fall in blood
concentrations. The persisting high concentrations can produce profound sedation, coma or respiratory depression (Walker et al., 1998).

If an IV bolus is given to a subject who is in effect exercising (e.g. during convulsive status), the three-compartment-model does not apply because the high rate of muscle blood flow brings it effectively into the central compartment (Shorvon, 1994). Drug concentrations therefore are unexpectedly low and do not decay biphasically. In this situation, drug disposition in the convulsive stage of SE may be very different from that expected on theoretical grounds, a possibility that has not been explored.

1.2.3 Hepatic metabolism

Almost all the AEDs used in SE are metabolised in the liver, via cytochrome P450 (CYP)-dependent microsomal and to a lesser extent non-microsomal enzymes (Conney et al., 1965; Fanelli et al., 1969; Liu et al., 1991). Metabolism comprises two phases: a degradation process (e.g. oxidation, hydrolysis, reduction) followed by a synthetic process (e.g. glucuronidation or sulphonation). Hepatic enzymes involved in the metabolism of many AEDs comprise CYP2C9, CYP2C19 and CYP3A4 and uridine diphosphate glucuronosyltransferase (Tanaka, 1999). Enzyme activity in both phases is often age-dependent (Herrlinger et al., 2001), can vary widely in a population due to genetic polymorphism (Meyer et al., 1997) and is affected by co-medication (induction or inhibition of enzyme activity) (Liu et al., 1995) and can be saturable (e.g. PHT) at dosages used in SE (Richens, 1979). In SE, impairment of hepatic function is not uncommon (and may be precipitated by therapy), and can profoundly alter drug metabolism (Shorvon, 1994). For some drugs (e.g. PHT and thiopentone), hepatic metabolism is saturable, and
above certain dosage rates drug concentrations will rise rapidly (Richens, 1979). The microsomal enzymes are often highly and rapidly induced by the massive drug exposure that is common in SE (Conney et al., 1965; Burba, 1968; Valerino et al., 1973). This induction may develop after only days of treatment and the drug dosages may need to be greatly increased.

Most drugs are metabolised to pharmacologically less active forms, but there are exceptions to this general rule. Active metabolites may complicate the assessment of the efficacy of an AED regimen, particularly if the pharmacokinetic parameters of the metabolites differ from those of the parent drug (Shorvon, 1994). DZP is converted to N-desmethyldiazepam, which has an antiepileptic activity about 30% of that of DZP, but which accumulates with much slower clearance, and thus during long-term therapy assumes considerable importance (Herman et al., 1996). With midazolam, in-vitro binding studies showed that the affinity of binding to the cerebral benzodiazepine receptor of the glucuronidated alpha-hydroxymidazolam metabolite was only about ten times weaker than that of midazolam or the unconjugated alpha-hydroxymidazolam metabolite. However, high concentrations of conjugated alpha-hydroxymidazolam cause prolonged sedative effect when concentrations of the unconjugated metabolite and the parent drug are below the therapeutic range (Bauer et al., 1995).

The half-life is the time taken for the drug concentration in the blood to fall by 50%. Where the blood concentration decay is biphasic, the half-life in the first phase is known as the distribution half-life and in the second as the $t\frac{1}{2}$. These characteristics are of critical importance in the drug treatment of SE, where large doses of lipid soluble drugs are being given intravenously over a long period of time. The shorter the distribution half-life, the more transient the initial effectiveness of the
drug is going to be; the longer the $t\frac{1}{2}$, the more likely is drug accumulation. The dangers of high cerebral concentrations and slow elimination are greater the longer the duration of therapy, which therefore requires careful drug concentration monitoring.

1.2.4 Clearance and excretion

The clearance of a drug is the rate of elimination divided by the concentration of the drug in the blood. Clearance depends on such factors as the rate of blood flow and hepatic enzyme capacity (for drugs metabolised in the liver). For some drugs, e.g. chlormethiazole, with rapid renal and hepatic elimination (Jostell et al., 1978), renal or hepatic blood flows are the major factors determining clearance. For such drugs, a fall in blood flow, common in SE, can result in a rapid and unpredictable rise in the blood concentration.

Most drugs in SE are excreted through the kidney only after metabolic transformation, a process that is directly dependent on glomerular filtration rates (Shorvon, 1994). This process is altered in SE only if severe renal disease is present or if cardiovascular disturbance greatly reduces renal blood flow.

1.3 Drugs used in the management of status epilepticus

The following section will review those drugs that are currently used in the management of SE in relation to their pharmacokinetics, neuropharmacokinetics, and efficacy in SE. The drugs will be reviewed in alphabetic order.
1.3.1 **Diazepam**

When DZP (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one; C$_{16}$H$_{13}$ClN$_{2}$O; molecular weight 284.8; Figure 1.1) was introduced into clinical practice in 1963 (Sternbach, 1980), a new pharmacological era for the management of SE was begun. DZP remains the drug of first choice for the treatment of a wide range of SE types in premonitory or early stages of SE (Shorvon, 1994). In addition, DZP has been used in the acute treatment of ongoing seizures (Schmidt, 1995).

DZP is very lipophilic and relatively insoluble in water. The parenteral solution is therefore prepared in propylene glycol and alcohol (Korttila et al., 1976) or in cremophor EL (for injection or rectal tube formulation), or as an emulsion.

*Figure 1.1. Structural formula of diazepam.*

![Structural formula of diazepam](image)

**Pharmacokinetics**

Following an IV injection, peak blood concentrations are reached within 3-15 minutes in volunteers (Baird et al., 1972; Hillestad et al., 1974) and sufficient cerebral concentrations are reached within about a minute. In contrast, IM injections produce variable concentrations (Gamble et al., 1975; Meberg et al., 1978), and C$_{max}$ may be lower and more delayed than after oral administration (Hillestad et al., 1974)
1974). Cmax is higher and produced more rapidly following administration of rectal solution of DZP than after either oral or IM administration. In adults, concentrations peak at about 20 minutes after rectal instillation (Remy et al., 1992). Absorption is faster in children, and effective blood concentration is usually reached within 5 minutes (Knudsen et al., 1992). Bioavailability after rectal administration is variable, in one study being as low as 50% (Magnussen et al., 1979). Bowel movement may also impede absorption and complicate therapy. Nevertheless, rectal instillation is a convenient alternative to IV injection.

A two-compartment model approximately describes the distribution of DZP in the acute phase after IV administration. After a single IV injection (5-10 mg), blood concentrations initially fall rapidly and then more slowly, from a high peak to sub-therapeutic values (below 200 ng/ml [0.7 μmol/L]) within 20 minutes (depending on Cmax). Only 3-5% remains in the brain tissue (Friedman et al., 1985). After repeated dosing, baseline levels are high because of the previous infusions and as the lipid stores are substantial, decay of concentration by redistribution does not occur. The much slower process of metabolism becomes largely responsible for the decline in blood concentrations. Thus repeated bolus injections produce high Cmax which persists, carrying an attendant risk of sudden and unexpected central nervous system (CNS) and cardiovascular depression. After an IV dose, the distribution half-life is about 60 minutes (reported values between 20-240 minutes; Magnussen et al., 1979), although blood concentration may rise paradoxically to a smaller peak 6-12 hours after a single IV injection, due to remobilisation of the drug and a build-up of N-desmethyldiazepam (Baird et al., 1972; Meberg et al., 1978).

The apparent volume of distribution of DZP is 0.8-2.6 L/kg (mean 1.1 L/kg), it is higher in children and in obese individuals (Abernethy et al., 1981). Both
DZP and its metabolite N-desmethyldiazepam are strongly protein-bound (90-99%). Binding is reduced in hepatic disease and neonates, but is otherwise unaffected by age (van der Kleijn et al., 1971).

DZP is metabolised by hepatic microsomal enzymes. The major metabolite is N-desmethyldiazepam, which has itself antiepileptic activity of about one third the potency of the parent drug (Mandelli et al., 1978). N-desmethyldiazepam has a longer t½, and if administration is prolonged this metabolite is responsible for a greater proportion of the antiepileptic action (steady state levels of DZP occur at about 7-8 days and N-desmethyldiazepam at about 15 days). N-desmethyldiazepam concentrations are generally of little significance in the emergency treatment of SE, unless the drug is given by continuous infusion for prolonged periods (Shorvon, 1994).

The t½ of DZP, which is not concentration-dependent, is between 18 and 100 hours (mean 20-40 hours), and is 30% shorter in patients receiving enzyme-inducing AEDs, shorter in infants (10 hours) and children (20 hours), and increased in the elderly (80 hours) and in the obese. In hepatic disease, the t½ is greatly increased to 99-164 hours (Andreasen et al., 1976; Klotz et al., 1977).

Less than 0.5% of DZP is excreted unchanged (Kaplan et al., 1973), 2.5-9% is excreted as N-desmethyldiazepam and the remainder as 3-hydroxy metabolites and their glucuronide conjugates. DZP clearance varies from 0.02-0.03 L/kg per minute but falls sharply with repeated dosing, and also is much lower in neonates and infants, and in renal and hepatic disease (Eadie et al., 1989; Schmidt, 1985).
Neuropharmacokinetics

Brain concentrations of DZP are about twice those in blood (van der Kleijn et al., 1983). The drug concentrates in white matter and brain stem structures rather than in grey matter or hippocampus. Both brain concentration and brain / blood concentration ratio fall rapidly in the first 60 minutes after infusion (in contrast to LZP), demonstrating the relative weak binding of DZP to benzodiazepine receptors. As the duration of action of DZP is relatively short, relapse after initial therapy is common, and longer term AED therapy is often required once the transient effects of DZP have worn off (Shorvon, 1994). DZP penetrates into the cerebrospinal fluid (CSF) compartment rapidly and CSF to serum ratio reaches equilibration within 10 minutes of intraperitoneal (IP) injection. CSF concentration of DZP was equivalent to the serum-unbound DZP concentration (Walker et al., 1998).

Efficacy in SE

With its rapid onset of action and efficacy against tonic-clonic, absence and partial SE, DZP is recognised as a drug of first choice in the treatment of SE (Schmidt, 1985). However, due to the rapid redistribution of DZP in the body, the efficacy in stopping SE should be maintained by repeated administration (carrying a risk of drug accumulation and respiratory stress) or replaced with a drug with longer lasting action, i.e. PHT.

1.3.2 Lorazepam

The first reported use of LZP (7-chloro-5-(2-chlorophenyl)-1,3-dihydro-3-hydroxy-2H-1,4-benzodiazepin-2-one; \( \text{C}_{15}\text{H}_{10}\text{Cl}_2\text{N}_2\text{O}_2 \); molecular weight 321.2; Figure 1.2) in clinical practice was in 1973. It is commonly used as an anxiolytic.
Nevertheless its efficacy in the treatment of SE has been widely recognised (Brown, 1982; Farrel, 1986; Homan et al., 1983; Lederman, 1984; Niedermeyer et al., 1985; Nourtsis, 1985).

LZP is a crystalline substance, insoluble in water, and unionised at physiological pH. It is only moderately lipid soluble, and much less so than DZP. The long duration of action and relative lack of redistribution for LZP confers substantial advantages over DZP (Homan et al., 1995).

Figure 1.2. Structural formula of lorazepam.

Pharmacokinetics

After an IV bolus injection, LZP distributes rapidly, with a distribution half-life of about 0.1-0.2 hours. The volume of distribution is 1.67 L/kg (Greenblatt et al., 2000), and it is 88-92% bound to plasma proteins.

LZP is metabolised by hepatic microsomal enzymes, largely by glucuronidisation, and unlike most other benzodiazepines, there are no active metabolites (Gerkens et al., 1981). The blood concentration of LZP is unaffected by even severe hepatic or renal disease (Morrison et al., 1984; Peppers, 1996). The t½ of LZP is 13.8 hours (Greenblatt et al., 2000). LZP is excreted via the renal route as
glucuronide derivative, and the renal clearance of the metabolite is 109 ml/minute (range 102-116 ml/minute).

**Neuropharmacokinetics**

The CSF concentration of LZP is about 10-15% of that in the blood, and this proportion does not vary with blood concentrations (Ochs et al., 1980; Aaltonen et al., 1980). In rats, cerebral concentrations are about 40-fold higher than free blood concentrations, and cerebral concentrations decline more slowly over time than blood concentrations (Walton et al., 1990b).

**Efficacy in SE**

Overall LZP is indicated in the early stage of SE only, and it is highly effective at all age groups (Shorvon, 1994). With its longer duration of action than DZP and small risk of accumulation, LZP is increasingly replacing DZP as the drug of choice in the early SE. Experimental studies have often shown LZP to have a greater efficacy than that of other benzodiazepine drugs, and a longer action than its blood t½ would predict (Valin et al., 1981).

**1.3.3 Phenytoin**

As PHT was investigated in this thesis, it is extensively reviewed in Section 1.7.1.
1.3.4 Propofol

Propofol (2.6-diisopropylphenol; C<sub>12</sub>H<sub>18</sub>O; molecular weight 178; Figure 1.3) is a short-acting IV anaesthetic and is relatively non-toxic. It is highly lipid soluble and slightly soluble in water.

Figure 1.3. Structural formula of propofol.

Propofol distribution follows a three-compartment model (Kanto et al., 1989) with a distribution half-life of 2-4 minutes (Cockshott et al., 1987). As it is extensively lipid soluble, its volume of distribution at steady state is large (9-17 L/kg; Kirkpatrick et al., 1988).

Propofol is extensively bound to plasma protein (97-98%), and extensively metabolised by hepatic P450 enzymes, to the glucuronide and 4-sulphate quinol derivative. The t½ of propofol is 30-60 minutes (beta phase), and 184-480 minutes (gamma phase) (Kanto et al., 1989). The total clearance is 1.5-2 L/minute (Cockshott et al., 1987). As this is higher than hepatic blood flow, it implies that the clearance of propofol is extrahepatic, although the mechanism is unclear. In patients with uncomplicated liver damage, the elimination phase is slightly prolonged (Servin et al., 1988).
Neuropharmacokinetics

Following IV infusion in rats, concentration of propofol in the brain increased and decreased expeditiously during and after infusion at anaesthetic dose (60 mg/kg per h) (Shyr et al., 1995). The mean blood-brain equilibration half-life was only 2.9 minutes (Kanto et al., 1989) and the extraction ratio across the brain was 0.85 (Upton et al., 2001). Following IV infusion of 15 mg/kg and 30 mg/kg propofol, the concentration of propofol in the brain, whole blood and plasma was 15.7 ± 1.9 and 39.4 ± 2.7 µg/ml, 4.5 ± 1.2 and 13.6 ± 1.3 µg/ml and 1.8 ± 0.5 and 5.1 ± 0.9 µg/ml respectively. Propofol distributed evenly in the brain and spinal cord during infusion, but there was a significant inter-individual variation (Shyr et al., 1995).

Efficacy in SE

Propofol is a highly effective drug with rapid onset of action and recovery is also very quick. It is particularly useful for the treatment of SE refractory to other AEDs (Merigian et al., 1995; Begemann et al., 2000).

1.3.5 Thiopentone Sodium

When conventional therapy proves ineffective in SE, it is the usual practice to induce anaesthesia in an attempt to control seizures. The barbiturate drugs, thiopentone and pentobarbitone, have the advantage of substantial antiepileptic action (Shorvon, 1994). However, their pharmacokinetic properties are problematic and the drugs are potentially highly toxic, and thus they are used with caution.
Thiopentone (5-ethylidihydro-5- (1-methylbutyl)-2-thioxo-4, 6(IH, 5H)-pyrimidinedione monosodium; C_{11}H_{17}N_{2}SNaO_{2}, molecular weight 264.3; Figure 1.4) is commonly used as an IV preparation in SE, unless anaesthesia is desired. It is a hygroscopic powder, alkaline in solution, highly soluble in lipid and also freely soluble in water (sodium salt). At pH 7.4, it is 39% ionised.

Figure 1.4. Structural formula of thiopentone sodium.

\[
\text{\begin{align*}
\text{O} & \quad \text{Na} \\
\text{CH}_3\text{CH}_2 & \\
\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2 & \\
\text{N} & \quad \text{S} \\
\text{N} & \quad \text{O}
\end{align*}}
\]

Pharmacokinetics

Thiopentone is used in SE only by IV infusion. IM injection may cause severe local injury, and sloughing of the skin may follow extravasation of the drug from a poorly positioned IV site (Shorvon, 1994). After IV injection, the distribution into tissue is very rapid (with a distribution half-life of 2.5 minutes) due to its high lipid solubility (Ghoneim et al., 1978).

The volume of distribution is 1.96 kg/L initially, though it is much greater on prolonged therapy, rising commonly to 5 kg/L or more (9.16 in one patient) (Turcant et al., 1985). In elderly patients the volume of distribution is the same (Ho et al., 1988). Thiopentone is 65-85% bound to plasma protein, but binding is significantly reduced in the elderly (51-60%; Ho et al., 1988).

Thiopentone is metabolised in the liver, by CYP isoenzymes, largely by phase I desulphuration and oxidation. Its metabolite, pentobarbitone has potent antiepileptic action. Thiopentone exhibits saturable pharmacokinetics (Bischoff et al.,
1968). Thus, at concentrations below 30 mg/L, the $t_{1/2}$ is 3-11 hours (Pandele et al., 1983; Russo et al., 1995; Le Corre et al., 1993). At a higher concentration or after prolonged therapy, it may increase to 18-36 hours (Turcant et al., 1985). Thiopentone elimination and total plasma clearance are not significantly different in patients with liver and kidney damage (Pandele et al., 1983; Christensen et al., 1983).

**Neuropharmacokinetics**

Animal studies comparing thiopentone in the posterior sagittal sinus and peripheral circulation reveal the equilibration half-life between the blood and the brain to be $0.67 \pm 0.07$, $0.57 \pm 0.03$ and $0.74 \pm 0.05$ minutes for the 250-, 500- and 750-mg doses, respectively, showing that the cerebral concentrations of thiopentone rapidly equilibrate with the afferent blood concentration (Upton et al., 1996). Indeed, unconsciousness occurs in 10-20 seconds (first pass of the bolus of drug through the brain), as the drug is taken up into the most vascular areas of the brain (grey matter first), and this uptake is flow-dependent (Shorvon, 1994). Brain microdialysate concentrations of both enantiomers increased from about 3% of corresponding plasma concentrations at 1 minute to 9% at 20 minutes. The highest thiopentone concentrations can be achieved at 20 minutes in CNS tissue, at 30 minutes in muscle and at 60 minutes in fat (Mather et al., 2000).

**Efficacy in SE**

Thiopentone is effective in halting seizures in patients with either partial or generalised SE refractory to even very large doses of other AEDs, including DZP (100-420 mg), PHT (1000-1750 mg), and in whom SE has continued for at least four
days (Brown et al., 1967; Partinen et al., 1981; Young et al., 1980; Orlowski et al., 1984).

1.4 Brain excitatory and inhibitory neurotransmitters in seizures

"Abnormal discharges are due to potentiation of excitatory mechanisms or to a failure of intrinsic cerebral inhibitory systems." -- Gowers, 1881.

Epileptic seizure can be thought of as paroxysmal hypersynchronous transient discharges in the brain that result from too much excitation or too little inhibition in the area in which the abnormal discharge starts. Excitation and inhibition of neurons may be mediated by many different neurotransmitters. Glutamate and gamma-aminobutyric acid (GABA) are now recognised as the principal excitatory and inhibitory neurotransmitters in the brain (Schwartz, 1988; Dickenson, 2001; Meldrum, 2002).

1.4.1 Neurochemistry of glutamate and gamma-aminobutyric acid

Glutamate

Neuronal glutamate may be produced from diverse origins as aspartate, glucose via pyruvate, the Krebs cycle and the transamination of alpha-oxoglutarate. It seems likely that most of the transmitter originates from the deamination of glutamine (Dickenson, 2001). After release, the high-affinity uptake sites (transporters) remove glutamate from the synapse, partly back into the nerve terminal or more probably into adjacent glial cells. In the latter, it is converted by glutamine synthetase into glutamine, which then passes back into the ECF to be taken up by the glutamate nerve terminal. This complex but very general biochemical process
provides very little opportunity for drug modification of glutamate synthesis or metabolism (Dickenson, 2001).

There are four types of glutamate receptors: N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA), kainate, and metabotropic (Dickenson, 2001). In most areas of CNS studied, the initial stage of excitatory synaptic transmission is a fast-depolarising response due to the release of glutamate and subsequent activation of AMPA receptors. Activation of the NMDA receptor produces slow prolonged neuronal depolarisation. Alteration in the transmission of neuronal information via NMDA receptors arise due to two main factors, the first being that the calcium influx through the channel produces large depolarisations, the second due to the unique profile of the receptor-channel complex, which requires various conditions for operation, and therefore is not necessarily involved in synaptic transmission at all times and under all circumstances. The release of excitatory amino acids is obviously needed but in addition, glycine is required as a co-agonist. Finally, an induced depolarisation of the neuron to relieve the resting voltage-dependent magnesium block of the channel is a prerequisite for activation of this complex. The NMDA receptor is therefore unique in that it is not only ligand-gated but also voltage-gated due to the channel block imparted by magnesium. Many kainate receptors are thought to be located presynaptically on terminals of neurons that release glutamate. Kainate receptors are therefore thought to be excitatory auto-receptors that enhance the release of glutamate and antagonists at this receptor should reduce excessive glutamate release. The metabotropic receptors are poorly understood so far but broadly, the Group I receptors are excitatory and Groups II and III are inhibitory and there is evidence for both pre- and postsynaptic locations of all groups of receptors (Dickenson, 2001).
**Gamma-aminobutyric acid**

GABA is an 'inhibitory' neurotransmitter in as much as its principal action is to cause membrane hyperpolarisation, thus reducing neuronal activity. GABA is located primarily in short-axon interneurons. The synthesis and metabolism of GABA is closely linked with that of glutamate and the citric acid or tricarboxylic acid cycle. GABA is formed within GABAergic axon terminals by transamination of alpha-ketoglutarate to glutamic acid, which is then decarboxylated by glutamic acid decarboxylase to GABA. Glutamic acid decarboxylase is found in several non-neuronal tissues (including ovary and pancreases) but within the CNS it is a specific marker of GABAergic neurons. Once released from a vesicle, GABA molecules are able to activate GABA receptors located on the pre- and post-synaptic membrane before rapidly diffusing out of the synaptic cleft. At the synaptic cleft, GABA is rapidly removed by uptake into both glial and pre-synaptic nerve terminals. The ultimate removal of GABA from the extracellular space, and the maintenance of a low extracellular GABA concentration, is achieved by the high affinity Na⁺ and Cl⁻ dependent uptake by GABA transporters (GAT-1, GAT-2, GAT-3 and BGT-1) into both GABAergic neurons and glial cells. This is a secondary active transport mechanism, but in this case GABA uptake is coupled to the movement of Na⁺ down its electrochemical gradient into the cell. Drugs that block the uptake of GABA may be beneficial in conditions of reduced GABA function, as they are likely to prolong the action of synaptically released GABA (Thompson et al., 1992). Once being removed to the presynaptic nerve terminal and glial cells, GABA is catabolized by GABA transaminase, producing glutamate and succinic semialdehyde. The latter is converted to succinic acid by succinic acid semialdehyde dehydrogenase and then re-
enters tricarboxylic acid cycle. This synthesis and metabolism of GABA is often referred to as the 'GABA-shunt'.

There are three types of GABA receptors: GABA_A, GABA_B and GABA_C. GABA_A receptors are the most widely expressed of the GABA receptors in the CNS and are found at the vast majority of GABAergic synapses. The GABA_A receptor complex is a pentameric heterooligomer that contains binding sites for GABA, barbiturates, benzodiazepines, picrotoxin, and neurosteroids. Binding of the two molecules of GABA to the receptor causes the opening of an integral transmembrane anion channel (Bormann et al., 1987). An increase in Cl⁻ conductance leads to an influx of Cl⁻ that results in membrane hyperpolarisation. This is the classic GABA-mediated inhibitory postsynaptic potential.

Both GABA_A and GABA_C receptors are ligand-gated ion channels that hyperpolarize the neuron by increasing inward chloride conductance and have a rapid inhibitory effect. GABA_B receptors are G protein-linked receptors that hyperpolarize the neuron by increasing potassium conductance. GABA_B receptors decrease calcium entry and have a slow inhibitory effect (Bowery et al., 2000). GABA_B receptors are present on both excitatory and inhibitory axon terminals. Activation is associated with a decrease in neurotransmitter release, and thus GABA_B agonist drugs, such as baclofen, under some circumstances may be antiepileptic (Swartzwelder et al., 1986) and in other experiment paradigms, pro-epileptic (Mott et al., 1989). GABA_C receptors are not affected by benzodiazepines, barbiturates or anaesthetics (Barnard et al., 1998; Bormann, 2000; Chebib et al., 2000).
1.4.2 Epileptic seizures and brain extracellular amino acids

Imbalance between excitatory and inhibitory neurotransmission in the CNS has been proposed as the mechanism underlying epileptogenesis (Meldrum, 1994). Using the microdialysis technique, changes in extracellular neurotransmitter amino acid concentrations were used as indirect evidence of neurochemical modifications during seizures in patients and animal models.

In epilepsy patients, seizures were associated with various degrees of increases of ECF glutamate and GABA (Carlson et al., 1992, During et al., 1993; Wilson et al., 1996). In During's study (1993) of patients with complex partial seizures and hippocampal sclerosis, there was a sustained increase in extracellular glutamate during seizures to potentially neurotoxic concentrations in the epileptogenic hippocampus. Moreover, the increase preceded seizure. GABA concentrations were unchanged before seizures, but increased during them, with a greater rise in the non-epileptogenic hippocampus. In Wilson's study (1996) the release of glutamate, aspartate, GABA and taurine increased from 2 to 4 fold during seizures, with glutamate release being the most markedly increased (4 fold). However, there was no consistent change in alanine, glutamine, serine, and threonine.

Numerous studies in animal seizure models produced various results. Amygdala kindling was associated with progressive, transient, stimulus-induced enhancement of ECF glutamate concentrations during the first minute post stimulus in both hippocampi (Ueda et al., 1994). In amygdala kindled rats intracerebral GABA substitution via GABA-releasing polymer matrices reduced seizure severity. This anticonvulsant effect was seen only when GABA concentrations were high (Kokaia et al., 1994). In the chronic rat model of epilepsy induced by kainic acid
injection into the hippocampus, release of glutamate, GABA and aspartate was increased up to 3, 10, 7 fold during seizures respectively (Wilson et al., 1996). In contrast, ECF amino acid concentration remained unchanged during afterdischarge (AD) induced by hippocampal electrical stimulation (Wilson et al., 1996). However Bruhn and co-workers (1992) reported different results in which glutamate remained unchanged while GABA concentration showed a 35% increase in the ECF. Intra-hippocampal administration of pilocarpine resulted in more than two-fold (232%) increase in extracellular glutamate and a simultaneous significant and sustained elevation of extracellular GABA concentrations to 173% (Khan et al., 1999). In genetically epilepsy-prone rat, high K+ infusion was associated with increased GABA, and glutamate release (Doretto et al., 1994; Lasley et al., 1994). Deep prepiriform cortex kindling stimulation caused significant increase in ECF glutamate and glycine concentrations in ventral hippocampus during kindling and up to 30 days after the last stimulation (Zhang et al., 1991). Soman induced seizures were also associated with increased glutamate concentrations in hippocampal CA1 and CA3 areas (Lallement et al., 1991). In seizures induced by bicuculline focal injection in the piriform cortex or picrotoxin systemic injection ECF glutamate concentrations did not change significantly either in the absence or in the presence of glutamate uptake inhibitors (dihydrokainate and 4,4'-diisothiocyanatostilbene-2,2' disulfonic acid) (Millan et al. 1991; Obrenovitch et al., 1996).

1.5 Neuronal damage from status epilepticus

Gross post-mortem examination of the brains of patients dying in acute SE often shows no obvious changes. In some cases, the brain is swollen and congested, and there may be scattered small haemorrhages. The hippocampus can be
swollen in the acute phase, especially in young children, and later become atrophic, shrunken and scarred. Severe atrophic changes may also be visible in the cerebellum (Shorvon 1994). Particular attention has been paid to damage in hippocampus since the early important study in 1880 by Sommer. Sommer documented, using Nissl-stained preparations of the brains of chronic epileptics, a pattern of damage that is now referred to as Ammon's horn sclerosis (or hippocampal sclerosis), in which gliosis and pyramidal cell loss occur, predominantly in the CA1 region of hippocampus. Subsequent investigators also recorded damage in other regions of cerebral neocortex, specific nuclei of the thalamus and in the cerebellum. Although the pathological findings are not in doubt, the relationship of the lesion to epilepsy, causal or consecutive, or both, remains uncertain and has been the subject of debate for many years. The link between hippocampal sclerosis and temporal lobe epilepsy (TLE) was not firmly appreciated until Sano and Malamud (1953) showed the Ammon's horn sclerosis in 29 of 50 institutionalised epileptic patients with associated anterior temporal spikes or spike-wave discharges. In a series of studies documenting the acute changes in SE (Norman, 1964; Ounsted et al., 1966), recent ischaemic hippocampal lesions were identified in 11 children dying in SE. The Ammon's horn was the most frequently damaged area. Amygdaloid and uncal changes were also found and more widespread neuronal damage in the neocortex, striatum, thalamus and cerebellum. The calcerine cortex was usually spared and there was patchy necrosis in other cortical and deep grey matter. Margerison and Corsellis (1966), in a detailed study of 55 institutionalised patients, found significant damage in the hippocampus in 65%, in the cerebellum in 45%, in the amygdala in 27%, in the thalamus in 25% and in the cortex in 22% of patients. It was concluded that hippocampal sclerosis was due to the ischaemic brain damage in early childhood
seizures or SE, and that SE-induced ischaemic hippocampal damage was probably the cause of the subsequent TLE. Corsellis and Bruton (1983) examined the brain of 8 children and 12 adults dying during or shortly after an episode of SE, and found swelling and almost complete loss of neurons in the Sommer section in all the children and three of the adults. These changes were recognised as the precursor of Ammon's horn sclerosis, in which the gliosis and atrophy would develop subsequently as the lesion matured. They also noted damage in the cerebella cortex and thalamus. The relative vulnerability of presubiculum, CA1 and CA3 regions to damage in human SE has been confirmed more recently by quantitative measurement of neuronal densities (DeGiorgio et al., 1992).

Although there is general agreement that the hippocampus is the brain region particularly severely and consistently affected in patients (especially in children) with histories of SE, other brain regions are also clearly affected. Widespread gliosis and neuronal loss in cortical and basal ganglion region were subsequently recorded in many pathological reports. The epileptogenic nature of cortical scarring was demonstrated by Penfield and Colleagues (Penfield, 1927; Penfield et al., 1940; Foerster et al., 1930). Norman (1964), Margerison (1966) and Corsellis (1983), although emphasising the Ammon's horn changes, also found in the acute phase of SE destructive cerebral changes, degeneration and loss of Purkinje cells and an acute glial reaction in the cerebellum, patchy massive neuronal destruction and gliosis in the thalamus, and similar damage in the corpus striatum. Acute neuronal necrosis was noted, especially in the middle cortical layers, stretching over a wide area of the cortical mantle in some cases, and only patchily distributed in others (Corsellis, 1983). In survivors of the acute lesion, gradual atrophy of the cerebellum was noted, in both the Purkinje cell and the granular layer,
and also widespread shrinkage, gliosis and neuronal loss in the neocortex and basal ganglion. In adults dying in SE the changes were much less striking than in children. In the worst of the 12 cases examined, acute hypoxic-ischaemic changes were noted, most marked in the hippocampus, but also affecting in a patchy fashion the neocortex, cerebellum and deep grey matter. In 7 of the 12 cases, no acute changes were found, although clear evidence of old scarring, heavy gliosis and neuronal loss, mainly in the hippocampus and cerebellum was evident.

The cellular changes after SE were summarised by Meldrum and Corsellis (1984). The nerve cell bodies were hardly visible with Nissl stain but were eosinophilic with haematoxylin and eosin stains. The cell outline was triangular, the nucleus was small and darkly stained and the nucleolus could no longer be identified. Many nerve cells disappeared, leaving only small tags of eosinophilic cytoplasm. In the cerebellum, the Purkinje cells were commonly affected, the cytoplasm lost its Nissl-staining substance and became eosinophilic, and the nucleus was dark and shrunken. A later change in affected parts of the brain was a microglial and astrocytic reaction, and eventually dense fibrous necrosis was laid down. The white matter showed diffuse but slight pallor. In adults similar pathological changes occurred but were usually less severe. All the above reports were of convulsive SE. A single paper describes three patients without pre-existing epilepsy who died 11-27 days after the onset of non-convulsive SE lasting 1-3 days (Wasterlain et al., 1993). In all three cases, there were changes similar to those outlined above, with neuronal loss in CA1, CA3 and hilar cells, and also in the amygdala, thalamus, cerebellum and cerebral cortex.

The clearest evidence that SE per se can result in cerebral damage has come from animal experiments. The classic studies of convulsive SE were carried
out in adolescent baboons by Meldrum and colleagues and have had a profound
influence on all subsequent work on SE (Meldrum et al., 1973a, 1973b, 1973c,
1974). In 10 baboons in whom generalised seizures lasting 82-299 minutes ischaemic
neuronal changes of the Spielmeyer type developed. The distribution of cell loss in
specific layers of neocortex, zones of hippocampus and portions of the basal
ganglion, and amongst certain cell types in cerebellum, correspond to the selective
pattern of vulnerability in the human brain after SE (Meldrum et al., 1973a). In order
to control the effect of convulsion to the neuronal changes the experiment were
repeated in paralysed and artificially ventilated baboons, in which hypotension,
acidosis, hypoxia and hypoglycaemia were prevented. Neuropathological
examination revealed a similar pattern of neocortical and hippocampal damage. Only
the cerebellar damage was totally prevented by paralysis and was thus thought to be
related to the hyperpyrexia and hypertension. It was concluded from these
experiments that 'ischaemic' change was not of vascular origin but due to impaired
 cellular mechanism.

To explore metabolic factors further, Meldrum and colleagues (1984)
studied mechanically ventilated Wistar rats in which oxygen and glucose
concentrations were maintained. Two hours of bicuculline induced SE produced
similar ischaemic changes, which suggested that the neuronal change was due to
oxidative mechanisms in cells with enhanced neuronal activity.

The differences between the cerebral damage induced by seizure activity,
hypoglycaemia and hypoxia have also been subject to intensive study (Siesjö, 1986;
Auer, 1988). All three insults cause cerebral damage that is distinct in degree,
distribution, timing and mechanism. Ischaemic damage was the least severe and
slowest to occur. In SE, cellular damage is maximal in the CA1, CA3 and CA4
regions of the hippocampus, layers III and IV of the cortex, cerebellum and the substantia nigra. At the cellular level, all three share similar excitotoxic processes, which probably form the final common pathway of cell damage; each cause glutamate release, the influx of calcium into cells, lipolysis with accumulation of free fatty acids, and proteolysis. The selective vulnerability of CA1 and CA3 in SE, maybe due to the high concentration of NMDA and kainate receptors distributed in this region.

1.6 Animal models of status epilepticus

SE is a surprisingly common and potentially life-threatening medical emergency. Nonetheless, much remains to be learned about this disorder. The mechanisms of the transition from a single seizure to SE are not known. Our understanding of the pathophysiological changes that cause SE to be progressively resistant to treatment is still incomplete. Researchers have only begun to understand the metabolic and pathologic consequences of sustained seizure activity; when permanent neuronal damage occurs during the course of SE is still not well worked out. Treatment of clinical SE is not always successful and there remains a need for the development of more effective and less toxic drugs for this purpose. The potential role of neuroprotective agents in the clinical management of SE is still unresolved, as is the cause of the differential susceptibility of young and adult brains to SE-induced neuronal damage.

The life-threatening nature of SE and ethical considerations impede the study on human subjects and prompt the use of experimental models. A number of models have been developed in intact animals based on systemic and focal administration of chemical convulsants and focal and generalised electrical
stimulation of the brain. More recently, slice preparations have been used to investigate recurrent and sustained seizure activity in isolated neuronal circuits.

1.6.1 SE induced by systemic administration of chemical convulsants

A variety of chemical convulsants have been administered systemically to induce various forms of experimental SE. Such agents act by either increasing neuronal excitation or decreasing neuronal inhibition (Table 1.1). Experimental models based on systemic administration of chemical convulsants have the advantage of simplicity — SE can be induced simply by parenteral administration of the convulsant agent. The disadvantage of such agents is their continuing presence once SE has been induced. Results may be confounded by drug interactions between the inducing agent and the experimental therapeutic agent.

1.6.2 SE induced by focal application of chemical convulsants

Most isolated seizures and episodes of SE in humans involve partial-onset seizures. Therefore experimental models of focal-onset seizures with secondary generalisation have particular utility in approximating the most common forms of human SE. Several such models have been developed. These include the modified alumina gel (Lockard et al., 1975), lithium chloride and bicuculline (Peterson et al., 1992), cobalt and homocysteine (Walton et al., 1988), domoic acid microinjection (Sharma et al., 1993), abrupt withdrawal of GABA infusion (Yang et al., 2001), and intra-amygdala injection of kainic acid (Kaijima et al., 1984) models.
Table 1.1. Mechanisms of chemical convulsants used to induce or exacerbate experimental SE (Treiman et al., 1997).

<table>
<thead>
<tr>
<th>Class / Mechanism</th>
<th>Chemical Convulsant</th>
</tr>
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<tbody>
<tr>
<td><strong>Excitatory agonists</strong></td>
<td></td>
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<tr>
<td>Glutamate agonism</td>
<td>NMDA</td>
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<tr>
<td></td>
<td>Dominic acid</td>
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<tr>
<td></td>
<td>Kainic acid</td>
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<tr>
<td></td>
<td>Quisqualic acid</td>
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<tr>
<td>Acetylcholine agonism</td>
<td>Pilocarpine ± lithium</td>
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<td></td>
<td>Soman</td>
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<tr>
<td><strong>Inhibitory antagonists</strong></td>
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<tr>
<td>GABA antagonism</td>
<td>Bicuculline</td>
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<tr>
<td></td>
<td>Pentylenetetrazol</td>
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<td></td>
<td>Picrotoxin</td>
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<tr>
<td>Adenosine antagonism</td>
<td>Methylxanthines</td>
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<tr>
<td><strong>Other agents</strong></td>
<td></td>
</tr>
<tr>
<td>NO synthase inhibition</td>
<td>L-nitroarginine</td>
</tr>
<tr>
<td>Na⁺ channel opening</td>
<td>Flurothyl</td>
</tr>
<tr>
<td>Pyridoxal phosphate antagonism</td>
<td>4-deoxypyridoxine</td>
</tr>
<tr>
<td>Glutamic acid decarboxylase inhibition</td>
<td>Mercaptoproprionic acid</td>
</tr>
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</table>

1.6.3 Electrogenic models

Electrogenic models of SE have the advantage that the inducing stimulus immediately ceases when the electrical stimulation is stopped. Thus response to drug therapy and evaluation of effects of SE are not compromised by an ongoing exogenous stimulus.
Taber et al. (1971) found that, by using an inter-stimulus interval of 1 minute applied to hippocampal electrodes, they could produce long-term, self-sustained, limbic and generalised SE in mice and subsequent deficits in an inhibitory avoidance task. Subsequently, Pinel and VanOot (1975) demonstrated that SE could be produced in rats when kindling stimuli were administered over several months. McIntyre et al. (1982) used a kindling-based electrical stimulation model of SE to induce partial-onset SE by applying 60 minutes of continuous electrical stimulation in the amygdala-kindled rats. Untreated rats showed massive gliosis and neuronal degeneration of the ipsilateral hemisphere.

Sloviter et al. (1981, 1983) used electrical stimulation of the perforant path, the main excitatory afferent to the hippocampus, to replicate limbic SE and to study seizure-induced neuronal damage independent from the metabolic effects associated with generalised convulsions. They demonstrated a persistent loss of recurrent inhibition and irreversibly damaged adjacent interneurons following granule cell seizure activity. GABA containing neurons survived, but there was a profound loss of adjacent somatostatin containing interneurons and mossy cells, thus suggesting that seizure-induced loss of basket cell-activating system may cause disinhibition. Vicedomini et al. (1987) used a stimulus current administered through electrodes implanted in the angular bundle or fimbria to induce self-sustained seizure activity that persisted after cessation of the electrical stimulation. In this model, the development of self-sustained seizure activity was essential for the production of neuronal damage. Indeed, as little as 17 minutes of self-sustained seizure activity was sufficient to cause at least some neuronal loss, whereas as many as 759 stimulus trains (2.1 hours of evoked synaptic activity) produced no evidence of neuronal degeneration. Lothman et al. (1989) described a model of self-sustained limbic SE
that exhibited the progressive EEG changes. In this model, as little as 30 minutes of
continuous focal electrical stimulation of hippocampus elicited SSSE that persisted
for many hours after discontinuing the electrical stimulation. Mazarati et al. (1998)
used the same model to study various AEDs. The study found that both DZP and
PHT had reduced efficacy the longer they were applied after electrical stimulation
and the longer the occurrence of electrical stimulation. Mazarati concluded that
AEDs, while highly effective on blocking the induction of SSSE, failed to affect its
maintenance. Halonen et al. (1996) prolonged the stimulation period to the perforant
path to 60 minutes and tested the new generation AED, tiagabine (TGB). TGB
treatment before the induction of SSSE reduced seizure severity as well as seizure-
induced damage to pyramidal cells in the hippocampus. The impairment of the
spatial memory associated with hippocampal damage was also reduced. Halonen et
al (1999, 2001) also tested remacemide in pre-treatment and lamotrigine (LTG) in
post-treatment of SSSE. Results indicated remacemide pre-treatment provided
moderate protective effect while LTG provided mild protective effect against SE-
induced neuronal damage (Halonen et al., 1999, 2001). Walker et al. (1999)
described a refractory model of SSSE induced by two hours continuous stimulation
of perforant path at 20 Hz. After cessation of electrical stimulation, behavioural and
EEG seizure activity is so severe that the animals have to rely on drug intervention so
as to survive. Even though AEDs stopped SE, there was severe neuronal loss in hilus,
CA1 and CA3, a pattern similar to those seen in human hippocampal sclerosis
(Sloviter et al, 1996; Cook et al., 2002). Holtkamp et al. (2001) used the same model
to compare the efficacy of DZP, PHT, pentobarbital and propofol in the treatment of
SE and proved it to be an ideal refractory SE model. In Holtkamp’s study 50 mg/kg
PHT administered at 10 minutes after electrical stimulation could not stop seizure
activity while the same dose had stopped SE after 30 minutes perforant path electrical stimulation (Mazarati et al., 1998). Only the anaesthetic agents propofol (50 mg/kg) and pentobarbital (60 mg/kg) stopped seizures completely whereas DZP (5 mg/kg) was effective in only 40% of animals.

The SSSE induced by different regimens of perforant path stimulation in Halonen’s and Walker's model consists of two phases: the induction phase during which seizures depend entirely on the persistence of the epileptogenic stimuli and stop if the stimulation is discontinued. The second, maintenance phase, is characterised by self-perpetuation of seizure activity in the absence of stimulation. A similar dependence on the epileptogenic agent during the initiation of SE, and the loss of this dependence later in the course of continuing seizures, have been described in the lithium-pilocarpine model of SE (Jope et al., 1986). The similarity of the data obtained from different animal models suggests that this is a common characteristic of SE. It also implies differential pharmacological profiles of the two phases and raises the question of the possible correlation between self-maintenance of seizures and resistance to AEDs. Indeed, clinical observations have suggested that the longer SE lasts, the more difficult it becomes to stop (Yaffe and Lowenstein, 1993). Experimental findings also showed a decrease in the efficacy of some AEDs as a function of the duration of SE induction (Morrisett et al., 1987).

1.6.4 In vitro models

In vitro models of SE have the advantage of simplicity and the possibility of isolating and manipulating specific components of the mechanistic process. As long as the neuronal circuitry in the models is sufficient to support repetitive electrographic seizures without complete recovery of the system to its baseline
physiology between seizures, in vitro models can be considered appropriate models with which to answer certain specific questions about mechanisms, consequences, and treatments of SE. Lowering of extracellular Mg^{2+} results in various forms of epileptiform activity in different parts of temporal lobe slices which contain neocortical areas such as areas Te2 or Te3, the entorhinal cortex, subiculum, hippocampal areas CA1 to CA3 and the dentate gyrus. This model is refractory to various AEDs therefore has been widely employed to study SE (Zhang et al., 1995; Sombati et al., 1995; Pal et al., 1999). Hippocampal and parahippocampal slices of post SE animals are useful in studying the excitatory and inhibitory postsynaptic potential with different manipulation and approaches (Lothman et al., 1995; Rafig et al., 1995).

1.7 Drugs investigated in this thesis

The following section will review those drugs that were investigated in this thesis in relation to their pharmacokinetics, neuropharmacokinetics, efficacy in SE and neuroprotective effects.

1.7.1 Phenytoin

PHT (5,5-diphenylhydantoin; C_{15}H_{12}N_{2}O_{2}; Molecular weight 252.3; Figure 1.5) was introduced for the treatment of epilepsy in 1938 (Yeo et al., 1978) and was first used in SE in 1958 (Carter and Gold, 1968). The success of PHT was one of the major pharmacological advances in treating neurological diseases and favourably altered the lives of many people with epilepsy worldwide.
PHT is a weak organic acid that is poorly soluble in water. The acid is essentially nonionised at pH 5.4, whereas at pH 7.4 (about 80% nonionised) the acid has a water solubility of 20.5 µg/g (25.2°C). The higher concentrations of PHT used for injection requires solutions with pH values around 12 (Glazko, 1989).

Figure 1.5. Structural formula of phenytoin.

Pharmacokinetics

In man, peak blood PHT concentrations are generally reached at between 4-8 hours after oral administration of a single dose (Dill et al., 1956). Jung (1980) found that the time to reach peak PHT concentrations increased progressively from 8.4 hours to 13.2 hours to 31.5 hours after 400, 800 and 1600 mg of PHT. PHT is absorbed more slowly when injected intramuscularly than when it is given orally (Dam et al., 1966) because of its poor water solubility, which makes it act as a repository preparation because of deposition of PHT crystals in the muscle. This causes hemorrhagic areas around the crystals (Wilensky et al., 1973). Thus when PHT must be given parentally, it should be administered intravenously.
Following absorption, PHT distributes freely in the body, reaching maximal volume of distribution (0.78 L/kg) within 15 minutes after absorption. Ninety percent (69-96%) of PHT is protein-bound (Lunde et al., 1970) and binding varies little with PHT plasma concentrations (Lunde et al., 1970). Newborn infants exhibit significantly lower protein binding of PHT than do adults (Ehrnebo et al., 1971).

PHT is metabolised in the liver, principally by hydroxylation, via CYP isoenzymes. Metabolising enzymes are potentially saturable, and exhibit genetically determined activity that varies widely between individuals (Andreasen et al., 1972). The major metabolites are 5-(4-hydroxyphenyl)-5-phenylhydantoin (p-HPPH) and a dihydrodiol metabolite, neither of which has significant antiepileptic activity (Chang et al., 1972; Browne et al., 1989b). PHT is excreted in urine and faeces mainly after metabolic transformation. Less than 5% of the total drug is excreted as the unmetabolised form in the urine; only a very small amount is excreted in this form in the faeces.

An important determinant of PHT plasma t½ is the dose of the drug. The dose dependence is best explained by saturation of a rate-limiting enzyme reaction in the metabolism of PHT. Saturation of biotransformation enzymes can also explain the observed fact that after high doses the decline in PHT concentration is not a first-order process, whereas it is at low dose.

**Neuropharmacokinetics**

In man, brain PHT concentrations are closely correlated to that in plasma. The brain / plasma ratio for PHT averages about 1.52, but white matter (2.73) contains approximately twice as much PHT as grey matter (1.73). The higher
concentrations of PHT in white matter are in part a result of high lipid content of this tissue, which is 2.5 times greater than that in grey matter of cerebral cortex (Masuda et al., 1979; Sherwin et al., 1973). PHT concentrations in the brain are 4-10 times higher than that in the CSF (Goldberg et al., 1978). The distribution of PHT in various brain regions was without difference (Scheyer et al., 1994a).

Animal studies revealed that upon intra-peritoneal administration to rats, PHT, rapidly enters the brain and reaches a Cmax in < 15 minutes in the CSF compartment (Lolin et al., 1994) and < 20 minutes in the brain ECF compartment (Walker et al., 1996). Transport of PHT across the BBB into the CSF compartment is rate limiting. Subsequently, PHT concentration in the CSF compartment immediately falls as the plasma concentration declines, while PHT concentrations in the brain ECF compartment plateaued for 40-60 minutes despite decreasing serum concentrations. The area under the concentration curve (AUC) for hippocampus was significantly greater than that for frontal cortex. The brain ECF / serum AUC ratio was 0.13 and 0.07 for hippocampus and frontal cortex respectively (Walker et al., 1996). Preferential accumulation of PHT in the superior and inferior colliculus, amygdala, and hippocampus, compared with 16 other areas of the brain was also observed in dogs and cats receiving PHT at an oral dose of 10 mg/kg for 14-16 months (Nakamura et al., 1966). In the rat brain, PHT t½ values for both hippocampus and frontal cortex were of the order of 4 h and were not statistically greater than the serum t½ (Walker et al., 1996). The PHT t½ in the CSF is significantly longer compared to that in the serum. Furthermore, the divergence between the blood and CSF compartments increases with chronic dosing which indicates accumulation kinetics (Lolin et al., 1994).
Efficacy in SE

PHT is highly effective in the treatment of SE and is indicated at the stage of established SE with high efficacy. Although over 20 new AEDs have been developed since PHT, PHT still remains the first line treatment option for SE. During the initial PHT infusion, 75% of 223 patients stopped seizing. A further 15% of patients stopped seizing soon after the infusion (Leppik et al., 1983). Effective cerebral levels of drug are maintained for many hours after PHT loading, and thus PHT loading commonly results in the permanent cessation of seizures. This is a distinct advantage over shorter-acting AEDs such as DZP. In man, PHT is effective when co-administered with DZP in treating SE, controlling 60% to 80% of seizures (Delgado-Escueta et al., 1982). In 43.6% of patients receiving PHT alone, all motor and encephalographic seizures ceased within 20 minutes of beginning IV infusion and did not recur during the next 40 minutes (Treiman et al., 1998). With its long duration of action, it can also be continued as maintenance therapy. However, it's an inconvenient drug to administer because of its strongly alkaline solution and the limitation on the speed of administration.

The anticonvulsant potency of PHT has also been studied in different SE models. In the transauricular electrical stimulation mice model, PHT proved capable of rapidly suppressing seizures after IV bolus injection (Honack et al., 1992). In the homocysteine thiolactone rat model with epileptogenic cobalt lesions, PHT is effective in arresting generalised convulsive SE (Walton et al., 1988, 1990a). PHT is also effective in aborting seizures in SE models induced by 30-60 minutes continuous electrical stimulation of the perforant path. The potency of PHT in this model declined the longer the duration of the electrical stimulation (30 minutes versus 60 minutes) and the later the drug administration was initiated after SE.
induction (10 minutes versus 40 minutes post-stimulation) (Mazarati et al., 1998). However, PHT is ineffective in kainic acid injection (Mecarelli et al., 1997) and lithium-pilocarpine systemic injection models (Morrisett et al., 1987; Sofia et al., 1993; Leite et al., 1995).

**Neuroprotective Effect**

PHT and other voltage-dependent sodium channel antagonists have been reported to be neuroprotective in both peripheral and central neurons. Weber and Taylor (1994) used rat hippocampal slices whereby the induction of ischaemia was possible. Pre-treatment with PHT prior to ischaemic induction prevented CA1 pyramidal cell damage. In a recent study by Schwartz and Fehlings (2001), the administration of PHT, riluzole or CNS5546A (a novel sodium channel blocker) to Wistar rats 15 minutes after the induction of compressive spinal cord injury, significantly enhanced residual tissue area at the injury epicentre compared with the control. Thus, administration of systemic sodium channel blockers can confer significant neuroprotection sparing both grey and white matter after in vivo spinal cord injury. Furthermore, retrograde venous perfusion of the spinal cord with PHT in a rabbit ischaemia model provided significant neuroprotection during prolonged spinal cord ischaemia (Gangemi et al., 2000). In a study of neocortical cultures deprived of oxygen and glucose to model ischaemic neuronal injury, cell death was measured by the release of lactate dehydrogenase (LDH) (Probert et al., 1997). LDH release was significantly reduced by treatments with PHT, tetrodotoxin, lidocaine, or the NMDA antagonist 3(2-carboxypiperazine-4-yl)propyl-1-phosphonic acid (CPP). Furthermore, combined treatments with tetrodotoxin and CPP were associated with pronounced reduction of LDH release. These results not only indicate that sodium
channel blocker is neuroprotective in neocortex cultures but also suggest that neuroprotection with such blockers may be due in part to inhibition of glutamate release.

1.7.2 Fosphenytoin

Although after IV or IM administration, PHT enters the central nervous system rapidly so that seizure control is prompt and effective, it has numerous undesirable characteristics. Firstly, PHT is poorly soluble, and consequently its formulation for the parental use requires the use of a vehicle of 40% propylene glycol and 10% alcohol adjusted to a pH of 12 with sodium hydroxide. IV administration of this formulation can result in hypotension, cardiac dysrhythmia and decreased pulse rate (Leppik et al., 1990; Earnest et al., 1983), which have been attributed to the propylene glycol vehicle (Leppik et al., 1990; Uthman & Wilder, 1989). Consequently, its administration has to be slow so as to avoid these complications (Leppik et al., 1983; Wilder et al., 1977). In addition, extravasation tends to cause local soft tissue and vein injury (Hayes et al., 1993; Kilarski et al., 1984; Rao et al., 1988; Weinstein, 1989). A further complication of IV PHT is the purple glove syndrome, which occurs in 25% of patients and is associated with oedema, limb discoloration and pain (O’Brien et al., 1998, 2001). FosPHT, (a disodium salt of 5, 5-diphenyl-3-[(phosphonooxy) methyl]-2-4-imidazolidinedione; molecular weight 406.24; Figure 1.6), the PHT pro-drug, has been developed to overcome these problematic characteristics.

Because FosPHT is water soluble, allowing formulation with a more physiologically compatible vehicle, it exhibits a number of advantages over PHT. It has better IV fluid compatibility and stability and therefore causes far less
cardiovascular, hepatic and renal adverse effects and can be administered at a faster rate. Furthermore, because FosPHT does not have a high pH value, it is associated with far less pain and burning at the infusion site and indeed minimal consequences occur after IV infiltration. These characteristics allow for a longer maintenance of IV infusion sites (Fierro et al., 1996). FosPHT is also well tolerated when administered intramuscularly (Ramsey et al., 1997; Pryor et al., 2001).

Figure 1.6. Structural formula of fosphenytoin.

Pharmacokinetics

Following IV administration of single FosPHT doses of 400 and 1200 mg PHT equivalent (PE), PHT Cmax increases in proportion to dose, but does not change appreciably with changes in infusion rate (FosPHT data sheet). With IV infusion at a rate of 75 mg PE/minute, the plasma Cmax of FosPHT and PHT is achieved by approximately 5.7 minutes and 42 minutes respectively (Leppik et al., 1990) and bioavailability of FosPHT is 100% (Browne et al., 1989a; Jamerson et al., 1990).

When administered as IM bolus, the plasma Cmax of FosPHT and PHT is achieved by approximately 36 minutes and 151 minutes respectively (Leppik et al., 1990). Plasma FosPHT concentration following IM administration are lower but
more sustained than those following IV administration. FosPHT is completely bioavailable following IM administration (Browne et al., 1989a).

The pharmacokinetics of FosPHT after oral administration has not been studied in man. In the rat, however, PHT plasma concentration profiles are characterised by a 85 % higher Cmax and a Tmax that is up to 2.6 hours shorter than that after the oral ingestion of a PHT suspension (Burstein et al., 1999).

FosPHT is extensively bound (95 to 99%) to plasma proteins, primarily albumin, and to a less extent alpha 1-acid glycoprotein (Lai et al., 1995). Binding is saturable and the concentration of albumin is the most important determinant for the plasma free fraction of derived PHT in man. FosPHT displaces PHT from its protein binding sites and increases the unbound fraction of PHT (up to 30%) during the period when conversion of FosPHT to PHT (approximately 0.5-1 hour post infusion) is occurring. The volume of distribution of FosPHT ranges from 0.032 and 0.048 L/kg (Gerber et al., 1988; Boucher et al., 1989).

FosPHT is rapidly converted on a molar-to-molar basis to PHT by phosphatase enzymes (Dasgupta et al., 2001). The conversion half-life of FosPHT after IV and IM administration averages 8.4 and 32.7 minutes, respectively (Leppik et al., 1990). PHT is subsequently metabolised and eliminated in the same way as that after PHT administration. In adult volunteers, serum AUC of PHT after FosPHT administration was not statistically different from that after PHT administration (Jamerson et al., 1990). A study of children (aged 5 - 18 years) indicated a similar pharmacokinetic profile to that observed in adults (Pellock, 1996).
**Neuropharmacokinetics**

In a study of rat whole brain PHT concentration, appearance of PHT in brain was delayed after FosPHT IP administration compared to that after PHT IP administration, with only negligible concentrations at 10 minutes postdose. During the 30 minutes postdose, brain PHT concentrations were significantly higher in PHT treated rats than they were for FosPHT treated rats. Subsequently, PHT brain concentrations were almost identical in both groups (Walton et al., 1999).

**Efficacy in SE**

In a series of 63 adults and 10 children with generalized convulsive SE, FosPHT terminated the convulsive SE in 95% (59 of 63 adults and 10 of 10 children) of patients (Fischer et al., 1996). There appears to be no significant difference in efficacy in children (aged 5 - 18 years) when compared with adults (aged 40 or less) (Pellock, 1996). Furthermore, IM FosPHT maintained seizure control when substituted for oral PHT (Wilder et al., 1996).

Animal studies have reported controversial findings. FosPHT has been shown to be less efficacious than PHT in a homocysteine thiolactone induced model of SE, but this has been explained on the basis that the time lag of conversion of FosPHT to PHT allows ongoing SE to become refractory (Walton et al., 1990a). In contrast, Loscher et al. (1998) found that PHT and FosPHT displayed similar anticonvulsant efficacy in amygdala kindled rats.

**Neuroprotective effect**

Data of the neuroprotective effect of FosPHT is very limited. Using a global ischaemia rat model, FosPHT has been reported to completely prevent
hippocampal CA1 neuronal loss (Chan et al., 1998). However, there are no reports of FosPHT neuroprotection in models of SE.

### 1.7.3 Tiagabine

TGB is a unique AED with a wholly novel mechanism of action. It is the first selective and specific GABA uptake inhibitor. Its antiepileptic effect is therefore the consequences of a rise in GABA content in brain extracellular space. It is licensed for adjunctive therapy of simple and complex partial seizures with/without secondary generalisation.

TGB hydrochloride [(R-n-(4,4-di(3-methyl-thien-2-yl)-but-3-enyl-nipecotic acid hydrochloride, molecular weight 412; Figure 1.7] is a nipecotic acid derivative linked by an aliphatic chain to a lipophilic anchor and consequently it can readily cross the BBB and enter the brain (Braestrup et al., 1990).

**Pharmacokinetics**

The pharmacokinetics of TGB in blood is linear and predictable, with plasma concentrations increasing in proportion to dose over the clinically relevant dosage range between 2-80 mg in adults (Gustavson et al., 1995; So et al., 1995; Samara et al., 1998; Ingversen et al., 2000) and 0.1mg/kg in children (Gustavson et al., 1997) respectively. After oral ingestion TGB is rapidly absorbed with plasma Cmax occurring within approximately 1 hour. Its bioavailability is 90% (Jansen et al., 1995). Interestingly, secondary peaks in the plasma concentration versus time profiles of TGB occur at approximately 10 hours after ingestion, suggesting enterohepatic recycling of TGB (Gustavson et al., 1995). Tmax and dose-adjusted
Cmax appeared to be dose-independent (Gustavson et al., 1995). Indeed multiple dose administration of TGB (6 or 12 mg/day for 14 days to healthy volunteers) was not associated with any significant TGB accumulation (Gustavson et al., 1995).

TGB is widely distributed throughout the body, including the brain. The mean apparent volume of distribution of TGB, as determined in patients on TGB monotherapy, is 52.0 L (range 15.6 - 137 L; Ingwersen et al., 2000). TGB is highly protein bound (96%), primarily to albumin and α1-acid glycoprotein (Elyas et al., 2002; Lau et al., 1997) and binding is concentration-independent (Cleton et al., 1999).

In healthy volunteers, single dose TGB at 4 mg is extensively metabolised, with only 2% of TGB excreted unchanged in urine (Bopp et al., 1993; Gustavson et al., 1995). Approximately 25% of TGB is excreted in urine and 63% in faeces as metabolites. TGB is oxidised in the liver by isoform CYP3A of the cytochrome P450 family of enzymes (Bopp et al., 1995), although a minor involvement of CYP1A2 is suggested (Samara et al., 1998). Thiophene ring oxidation results in the formation of two 5-oxo TGB isomers (E-5 and Z-5) and these metabolites appear to be the prominent metabolites in plasma of man, and numerous animal species, and are the major metabolites (60%) in urine (Bopp et al., 1993).
faeces the 5-oxo metabolites of TGB represent 8% of TGB dose. However, two additional metabolites found in faeces and representing 40% of TGB dose, have yet to be identified.

The plasma t1/2 of TGB is dose-independent and is 4.5-8.1 hours (3.8 - 4.9 hours with enzyme inducing AEDs) (Gustavson et al., 1995; So et al., 1995). There is no difference between age groups. Plasma clearance of TGB is 12.8 L/h (Samara et al., 1998; Ingwersen et al., 2000). TGB metabolism is impaired in patients with liver damage (Lau et al., 1997) but unaffected in patients with renal damage.

Neuropharmacokinetics

Regional distribution study of nine brain regions using [3H] TGB in homogenates of frozen post-mortem human brain showed that the binding was heterogeneous, with the highest binding in frontal cortex and parietal cortex and the lowest binding in nucleus caudatus and putamen (Eriksson et al., 1999). In rat brain, the autoradiographic distribution of [3H] TGB binding sites is also heterogeneously distributed. The highest density of [3H] TGB binding sites occur in the cerebral cortex, mammillary body, globus pallidus, substantia nigra pars reticulata, hippocampus, dorsal raphe, superior collicullus (out layer) and cerebellum (Suzdak et al., 1994). TGB readily penetrated BBB of a patient with epilepsy within an hour of oral administration as evidenced by rise of GABA concentration in ECF compartment (During et al., 1992).
Efficacy in SE

The first clinical study of the efficacy of TGB comprised a placebo-controlled enrichment design and it revealed a significant reduction in mean frequency of complex partial seizures and secondarily generalised tonic-clonic seizures (Richens et al., 1992). A subsequent double blind, parallel group trial revealed that TGB reduced complex partial seizure in dose-related manner (Rowan et al., 1993). Similar efficacy was observed in children (aged 4-17) (Kmiec et al., 2000).

TGB is effective in stopping or preventing seizures in perforant path stimulation (Halonen et al., 1996) and homocysteine thiolactone models of SE (Walton et al., 1994). In the perforant path stimulation model, subchronic treatment with TGB (via Alzet pumps, 50 mg/kg/day) completely prevented the appearance of generalised clonic seizures during stimulation (Halonen et al., 1996). In the homocysteine thiolactone model, TGB was successful in controlling generalised convulsive SE at a median effective dose of 8.3 mg/kg (Walton et al., 1994).

Neuroprotective effect

Studies of ischaemic neurons suggests that enhancement of neuronal inhibition with a GABA uptake inhibitor may be neuroprotective. In a gerbil ischaemia model, TGB (45mg/kg) significantly reduced the necrosis in the CA1 pyramidal cell layer (Inglefield et al., 1995). TGB also significantly reduced the ischaemia-induced elevation of glutamate concentrations in area CA1 during the post-ischaemic period when GABA concentrations were elevated. However the neuroprotective effect after ischaemia was attributed largely to the mild hypothermia caused by TGB. In the perforant path model of SE, subchronic treatment prior to SE
with TGB 50mg/kg via subcutaneous Alzet pumps reduced the loss of pyramidal cells in the CA3c and CA1 fields of the hippocampus (Halonen et al., 1996).

1.7.4 Topiramate

Topiramate (TPM, 2,3:4,5-bis-O-(1-methylethylidene)-beta-fructopyranose sulfamate, molecular weight 339.37; Figure 1.8), a white crystalline powder with a solubility of 9.8 mg/ml at pH 6.7, is licensed for adjunctive therapy of partial onset seizures, as well as generalised tonic-clonic seizures of non-focal origin in adults and children and drop attacks in patients with Lennox-Gastaut syndrome (Sachdeo, 1998; Glauser, 1999). The mechanism of action is multifactorial and includes inhibition of voltage-dependent sodium channels, facilitation of GABAergic inhibition, antagonism of excitatory transmission via the AMPA receptor and inhibition of carbonic anhydrase (Perucca, 1997).

Figure 1.8. Structural formula of topiramate.

Pharmacokinetics

TPM is well absorbed from the gastrointestinal tract (Nayak et al., 1994), with plasma Cmax attained by 2-3 hours (Doose et al., 1995). Oral bioavailability is in the order of 80-95% and TPM distributes into all tissues, including the brain.
(Nayak et al., 1994). The apparent volume of distribution is about 0.6-0.8 L/kg. In healthy volunteers, 55-60% of the administered dose is excreted unchanged in urine. Metabolic pathways involving hydroxylation and hydrolysis account for less than 40% of the drug clearance. Metabolic elimination however becomes the main determinant of drug clearance in epileptic patients who are receiving concurrent enzyme inducing AEDs such as PHT, barbiturates or carbamazepine (CBZ). In these patients, the fraction of the dose excreted unchanged in urine falls to 30%, compared with 55% in non-comedicated patients (Sachdeo et al., 1996). In subjects not receiving enzyme-inducing drugs, the $t\frac{1}{2}$ of TPM is in the order of 20-30 hours (Easterling et al., 1988). In patients taking enzyme-inducing AEDs, the $t\frac{1}{2}$ is significantly shorter and is of the order of approximately 12 hours. Total clearance values are 20-30 ml/minute in healthy volunteers (Easterling et al., 1988) and 50 ml/minute in patients co-medicated with enzyme-inducers (Sachdeo et al., 1996).

**Neuropharmacokinetics**

Studies of TPM in the CSF and plasma indicated that there was a close correlation between the plasma and the CSF concentration for both the total and unbound concentrations of TPM in man (Lindberger et al., 1999; Christensen et al., 2001). The free TPM concentration in plasma was not different from the free TPM concentration in CSF. The unbound fraction of TPM was 84% in plasma and 97% in CSF. The median CSF / plasma ratio of total TPM was 0.85. The CSF / plasma ratios showed little variation and were independent of the plasma level for both the total and the unbound levels. There does not seem to be a saturable carrier mechanism restricting TPM transport across the BBB (Christensen et al., 2001). However, there are no reports of the distribution of TPM in the brain.
Efficacy in SE

Clinical trials showed that all seizure types (simple partial, complex partial and secondarily generalised) were significantly reduced (43% - 58%) by TPM (Ben-Menachem, 1995b).

TPM is associated with a broad spectrum of anticonvulsant activity in seizure models (Maryanoff et al., 1989; Ben-Menachem, 1995a). Models in which it is effective include the maximal electrical shock test (MES) in mice and rats (Kimishima et al., 1992; Shank et al., 1994), the genetically seizure-prone DBA/2 mice (Nakamura et al., 1994), the spontaneously epileptic rats (Nakamura et al., 1994), and the amygdala kindled rats (Kimishima et al., 1992; Wauquier et al., 1996). In the MES test, the potency of TPM after oral administration is comparable to that of CBZ, PB and PHT and greater than that of VPA. TPM is inactive or weakly active in a number of chemically induced seizure models in rodents, including the clonic or tonic seizures induced by picrotoxin, pentylenetetrazol, bicuculline and strychnine (Shank et al., 1994). However, there has been no data regarding its efficacy in SE models.

Neuroprotective effect

A study of developing rats with neonatal seizures and SE following a 4-week TPM treatment reported that TPM offered no protection from neuronal loss or mossy fibre sprouting although TPM did provide moderate protection of cognitive function (Cha et al., 2002). In contrast, in adult rats with limbic SE induced by hippocampal electrical stimulation, 20-80 mg/kg TPM administered after SE attenuated hippocampal neuronal injury (Niebauer et al., 1999). TPM has also been reported to be neuroprotective in the cerebral ischaemic rat model (Yang et al., 1998,
2000), in which 20 mg/kg TPM significantly attenuated brain infarction volume and significantly improved the neurobehavioural score. Neuroprotection was attributed to the inhibition of TPM on the voltage-dependent sodium channel, which therefore lead to suppressive effect on glutamate release from the synapse.

1.8 The status epilepticus model used in this thesis

Electrogenic models of SE are considered superior to those models induced by chemical administration since the latter requires introduction of a foreign substance into the brain. The consequence is that the foreign substance persists in the brain, interferes with the interior system and therefore interpretation of results becomes more complicated.

Among many electrogenic models, the perforant path stimulation model has a number of advantages. Firstly, it involves stimulation of the main excitatory pathway to the hippocampus (Sloviter, 1981), which is a key element involved in initiating and maintaining the limbic SE (Lothman et al., 1989). Secondly, the time course in inducing limbic SE is easy to predict by observing the presence of synchronous, stimulus-independent electrographic seizure activity during stimulation. Thirdly as it does not involve kindling prior to the SE induction, the process of SE induction is relatively easy. Lastly the limbic SE is associated with far less metabolic disturbance which is a common consequence of the generalised convulsion and which may complicate the interpretation of results (Sloviter et al., 1981, 1983).

There are different regimens for perforant path stimulation to induce SSSE. Some regimens use 30 minutes or 60 minutes continuous 2 Hz electrical stimulation, together with 20 Hz intermittent electrical stimulation (Mazarati et al.,
Other regimens use continuous 60 minutes 20 Hz stimulation (Halonen et al., 1996; Pitkanen et al., 1996), or 2-hour continuous 20 Hz electrical stimulation (Walker et al., 1999). The longer the stimulation lasts, the more severe the SSSE (Mazarati et al., 1998). This is evidenced by previous studies that when perforant path stimulation was prolonged from 30 minutes to 60 minutes, the anticonvulsant efficacy of PHT significantly decreased (Mazarati et al., 1998). Furthermore, when perforant path stimulation was increased to 120 minutes, PHT completely lost its efficacy (Holtkamp et al., 2001).

Among these different stimulation regimens, the one described by Walker et al. (1999) displays the most persisting electroencephalographic and behavioural seizure activity upon completion of the electrical stimulation. These characteristics closely resemble those seen in human SE. Furthermore, the model is significantly severe and refractory so as to enable the study of drug treatments of refractory SE, which resembles human refractory SE. Thus the model described by Walker et al. (1999) was chosen in this study.

1.9 Aims of this thesis

Using an animal model that allows the temporal (blood) pharmacokinetics and central (CSF, ECF) brain neuropharmacokinetics of a drug and an electrogenic model of SE, this thesis has sought to investigate various aspects of AEDs used in the treatment of SE.

1) The blood pharmacokinetics and CSF and ECF neuropharmacokinetics of PHT after IV PHT were compared to those of derived PHT after IV FosPHT.
2) The blood pharmacokinetics and CSF and ECF neuropharmacokinetics of TGB after IP administration were determined.

3) Electrographic and histological changes in the perforant path stimulation induced SE model were characterised and the role of excitatory and inhibitory neurotransmitters at different stages of SSSE in this model was explored.

4) The antiepileptic characteristics of FosPHT, TGB and TPM in this SE model were determined and compared.

5) The neuroprotective properties of these drugs were investigated and compared by use of histological determinants.
2 MATERIALS AND METHODS

2.1 Animals

Male Sprague-Dawley rats weighing 280-350g were used in all studies. Rats were purchased from an accredited supplier (Tuck & Son Ltd, Essex, UK) and were housed in groups of 3 in contiguous cages and fed ad libitum on normal laboratory diet (SDS R and M number 1 expanded, Scientific Dietary Services, Witham, Essex, UK) and water. A 12-hour light-dark cycle (lights on 06.00 hour) and an ambient temperature of 25 ºC were maintained. All rats were allowed to acclimatise to their new environment for at least 48 hours prior to experimentation. All animal procedures strictly followed Home Office regulations and were performed in accordance with the Animal (Scientific Procedures) Act 1986.

2.2 Catheters / probes construction

2.2.1 Blood catheter construction

Silastic tubing (STHT-C-025 0.25 mm ID x 0.47 mm OD, Osteotec Ltd, Dorset, UK) was used to construct the blood catheter. Blood catheters were 10cm in length. One end of the catheter was cut into wedge-shape so as to ensure the smooth placement into blood vessels.

2.2.2 CSF catheter construction

The CSF catheter comprised three parts (Figure 2.1).
The outer part was made from polythene tubing (0.76 mm ID, 1.22 mm OD, Scientific Laboratories Supplies Ltd, England, UK). The tubing was cut to 2 cm long, with the bottom part enlarged by gentle heating to produce a bouton.

![Figure 2.1. Illustration of a schematic CSF catheter.](image)

The middle part of the CSF catheter was also made from polythene tubing (0.28 mm ID, 0.61 mm OD, Scientific Laboratories Supplies Ltd, England, UK), which was cut to 8 cm in length, with the tip bent to 60° and cut into wedge-shape.

The inner part of the CSF catheter comprised of a piece of 37-G enamelled copper wire (Scientific Wire Co., London, England, UK), which went
through the middle part of the CSF catheter. The wire served to provide rigidity to the catheter and to ease puncturing the dura during catheter implantation.

### 2.2.3 Microdialysis probe construction

Concentric microdialysis probes were constructed as follows. Two vitreous silica tubings (SGE, Milton Keynes, England) were inserted into a 18-mm long 24-G stainless steel tubing, with the longer tubing sticking out of both ends of stainless steel tubing and the shorter one sticking out of one end of the stainless steel tubing (Figure 2.2). A piece of filtral 12 dialysis membrane (6 mm in length, 200 μm in diameter; Hospal, Rugby, UK) was inserted into one end of the stainless steel tubing where only the longer silica tubing protruded. The dialysis membrane encased the silica tubing that protruded out of the stainless steel tubing. Subsequently two pieces of 6-mm long polythene tubing (0.28 mm ID, 0.61 mm OD) were attached to these two silica tubing. One tubing served as the inlet tubing, and the other as outlet. Quick set epoxy adhesive (RS Components, Northants, UK) was used to seal all joints. Wax was then melted and applied to one end of the stainless steel tubing so as to create a wax ball. This wax ball served to provide an extra area for holding the dialysis probe and easing the placement. After the wax and epoxy resin was set, the inlet polythene tubing was attached to a needle and syringe filled with saline. Pressure was then applied to the syringe so as to allow saline to go through the microdialysis probe slowly. This served to check the patency of the probe and ensure that there was no leakage at any of the joints.


2.2.4 Electrode construction

All parts, unless otherwise specified, were purchased from Plastics One Inc via Semat Technical, Herts, UK. Wire was purchased from Advent Research Materials, Suffolk, UK. Bipolar stimulation electrodes were constructed from two 125-μm Teflon-coated stainless steel wires twisted together, which were then attached to gold female connectors either by franking or soldering. The gold connectors could be mounted in a six-hole plastic pedestal along with the connectors for the recording electrode and earth. The tips of the stimulation electrode were cut
such that they were separated by 0.5 mm. A single 250-µm Teflon-coated stainless steel wire attached to a gold female was used as the recording electrode. Earth was a subcutaneous silver wire, which in freely moving rats was connected to an anchor screw on the skull.

### 2.2.5 Microdialysis probe-electrode construction

The microdialysis probe and recording electrode were fixed together with superglue (Figure 2.3).

*Figure 2.3. Schematic illustration of a microdialysis probe attached to a recording electrode. The actual area for microdialysis is 2mm in length and 200 µm in diameter. The distance between the tip of the recording electrode and the microdialysis probe is 0.5-1mm.*
2.3 Surgical procedures

In the kinetic studies, a well established freely behaving rat model (Patsalos et al., 1992; Lolin et al., 1994; Walker et al., 1996, 2000) was used to investigate the concurrent, temporal correlation between the kinetics in the blood, CSF and brain ECF of the various drugs.

2.3.1 Blood catheter implantation

Rats were anaesthetised by inhalation of 4% halothane (Merial Animal Health Ltd, Dublin, Ireland) and with oxygen flow rate at 4 L/minute. Anaesthesia was maintained during surgery with continuous halothane inhalation of 1.5% and with oxygen flow rate of 1 L/minute. Rats were placed on the surgery table on their back, with four limbs fixed with adhesive tape. Hair on head and chest was shaved. A 2.5-cm long incision was made in the midline between the collarbone and the sterna bone, and the right external jugular vein was exposed by blunt dissection. After removing the tissue surrounding the vein with a pair of tweezers, three silk threads (black braided silk suture, Davis & Geek, Hampshire, England, UK) were passed under the vein caudally, medially, and rostrally. The caudal thread was ligated. Using a pair of fine scissors, a tiny incision was made in the vein just below the medial thread. The catheter, which was filled with saline, was inserted into incision until it reached the right atrium (3.5 cm for rats weighing < 300g and 4 cm for rats weighing > 300g). The other two threads were used to secure the catheter within the vein and the caudal thread was used to secure the positioning of the exteriorised portion of the catheter. Patency of the tubing was tested before 90 µl of heparin (100 u/ml, heparin doses are indicated in international unit throughout this thesis) were infused into the catheter. The free end of the tubing was sealed with a small metal pin.
The rat was then placed on its chest and a sagittal incision was made at the top of the head. A subcutaneous tunnel from the right external jugular vein to the incision at the top of the head was exploited by a pair of tweezers. The blood catheter followed through the tunnel and emerged out of the incision on the head. The rat was sutured on the chest. A small quantity of well-mixed dental acrylic (Dentply Detrey, Dentsply Limited, Surrey, England) paste was applied to the top of the head to seal the incision and to fix the blood catheter firmly onto the top of the rat head. This served to prevent the rat from displacing the catheter by scratching. Subsequently the animal was housed individually in a Perspex cage and allowed to recover for two days.

2.3.2 CSF catheter implantation

Rats were anaesthetised as previously described and their hair on head and chest was shaved. The rats were placed in a stereotaxic frame (Stoelting Co., Woodlane, IL, USA) and fixed by use of ear bars and teeth bar which served to keep the head level. A 1.5-cm midline incision was made 5 mm caudal to the external occipital crest and the skin and the atlantooccipital membrane were retracted. Three burr holes were made, two at either side of the skull (3mm lateral and 4mm caudal to lambda) for the placement of stainless-steel anchor screws, the last one at the middle line (0.5 mm caudal to the external occipital crest and at an angle of 45°) for catheter insertion into the cisterna magna (a relatively large CSF compartment above the cerebellum). The CSF catheter was gently inserted into the hole until the button rested on the skull surface. The copper wire was subsequently withdrawn and the catheter fixed in place with dental acrylic. CSF was introduced gently into catheter by a slight negative pressure using a syringe. The CSF catheter was then cut to
approximately 6 cm, with the free end heat-sealed, and the skin was sutured. After the CSF catheter implantation, the rats were housed individually in Perspex cages and allowed at least 48 hours to recover before further experimentation.

**2.3.3 In vitro recovery procedure for microdialysis probes**

Each microdialysis probe was placed in the recovery solution, which comprised 100 ml artificial CSF (artificial CSF composition in mM: NaCl 125, KCl 2.5, MgCl₂ 1.18, and CaCl₂ 1.26) and drug solution (TGB or PHT) to be studied. The concentration of TGB and PHT in the recovery solution were chosen to match their estimated concentration in the CSF or brain ECF after correction of microdialysis probe recovery. The inlet polythene tubing of the microdialysis probe was attached to a piece of 50 cm long polythene tubing (0.28 mm ID, 0.61 mm OD) that was connected to a needle and syringe, which was placed on the microinjection pump (Harvard Apparatus, South Natick, Mass., USA). The syringe and tubing was filled with artificial CSF. The microdialysis probe was lowered into the recovery fluid at 37°C.

Before starting the experiment, the microdialysis probe was infused with artificial CSF at 2 μl/minute for 30 minutes and the recovered fluid discarded. Then the solution from the outlet tubing of the microdialysis probe was collected every 15 minutes at the flow rate of 2 μl/minute. The solution was collected in 0.5-ml polyethylene tubes and stored at -20 °C until analysis.

After completion of sampling, the microdialysis probe was flushed with distilled water at 2 μl/minute for 30 minutes and the inlet and outlet polythene tubing sealed with a fine stainless steel pin. The microdialysis probe was then stored in distilled water until probe implantation, which was within 24 hours.
2.3.4 Microdialysis probe implantation

Rats were anaesthetised using halothane 1-2%. The head was fixed onto a stereotaxic frame using ear bars and the teeth bar and the skull was kept level as in the Atlas described by Paxinos and Watson (1986). Hair on the head was shaved. An incision was made at the middle line on the top of the head, and the subcutaneous tissue was removed. Altogether four burr holes were made on the rat's skull. Two for anchor screws and the other two holes were for probe implantation in the hippocampus (from bregma 5.6 mm posterior, 5 mm lateral) and frontal cortex (from bregma 2.5 mm anterior, 1.5 mm lateral) according to the atlas of Paxinos and Watson (1986). The two anchor screws were first placed prior to microdialysis probe implantation. Any blood on skull and inside the burr holes was carefully cleaned by uses of tissue scrubs. The two microdialysis probes were slowly placed into position using the adjustment screw on the stereotaxic frame until the required depth (hippocampus probe to 8.2 mm, frontal cortex probe to 5.5 mm) was achieved. Dental acrylic paste was applied to surround the stainless steel part of the probes and burr holes so as to fix the microdialysis probes firmly on the skull. Then the skin was sutured and the rats were housed individually in Perspex cages and allowed to recover.

2.3.5 Electrode implantation

The rat was anaesthetised and the head was fixed as described in section 2.3.4. Two burr holes were made stereotaxically at the right hemisphere for the recording electrode (co-ordinates 2.5 mm lateral, 4 mm caudal from bregma) and the stimulation electrode (co-ordinates 4.4 mm lateral, and 8.0 mm caudal from bregma). Three other burr holes were made at either sides of the skull for placement of anchor
screws. A piece of 10-cm long silver wire was attached to one of the anchor screws, the other end of the silver wire was left in the subcutaneous tissue on the skull. This wire was used as the earth electrode. The stimulation electrode was advanced 3mm ventrally form the dura into the angular bundle for stimulation of the perforant path and the recording electrode advanced 4-5 mm ventrally from the dura into the dentate granular cell layer. Subsequently the depth of both the recording and stimulation electrodes was adjusted to maximise the slope of the field potential. All the electrodes were fixed in place with dental acrylate and three skull anchor screws. The location of the electrodes was later verified histologically, and only the rats with correct electrode placements were included in the analysis. The animals were placed in separate Perspex cages individually and allowed to recover fully from anaesthesia (at least 48 hours before further experiments).

2.3.6 Electrode-microdialysis probe implantation

The combined electrode-microdialysis probe was implanted using the same procedure as for microdialysis probe implantation in section 2.3.5. However implantation occurred in the hippocampus only.

2.4 Sample collection

2.4.1 Blood sample collection

On the sampling day, the metal pin sealing the tubing was removed and tubing patency was checked. The blood catheter was attached to a piece of polythene tubing (0.58 mm ID, 0.96 mm OD, Scientific Laboratories Supplies Ltd, England, UK) of 50 cm in length. The cannula was filled with heparinized saline (10 u/ml) and
attached to a 23-G needle and a 1-ml syringe. Venous blood was slowly withdrawn by applying slight negative pressure in the syringe. The needle was closed using a small cap and a new syringe was attached. Blood was collected (200 μl) at regular intervals of 15, 30 or 60 minutes over six hours after drug administration. In order to prevent hypovolemia, an equivalent volume of heparinized saline (10 μl/ml) was administered after each blood withdrawal to the rats via the cannula. The maximum volume of blood withdrawn did not exceed 2.5 ml over the whole sampling period. In the study of FosPHT and PHT kinetics, in order to prevent in vitro FosPHT conversion to PHT, 10 μl of 0.55 M ethylenediamine tetraacetic acid (Aldrich Chemical Company, Milwaukee, USA) at pH 14 was added to each sample collection tube.

Blood samples were subsequently centrifuged at 10,000rpm (Abbott Micro-centrifuge, Abott, Maidenhead, UK) for 5 minutes at 25 °C before the supernatant was stored in 0.5-ml centrifuge tubes at -20 °C until analysis.

2.4.2 CSF sample collection

The sampling of the CSF was achieved by connecting the implanted catheter to a cannula attached to a 23-G needle and 1-ml syringe. The cannula consisted of 24-cm long polythene tubing (0.28mm ID, 0.61mm OD, Scientific Laboratories Supplies Ltd, England), corresponding to a volume of 20 μl, and attached to a 15-cm long polythene tubing (0.58 mm ID, 0.96 mm OD). The cannula was disconnected from the catheter between sampling, and CSF samples were dispensed into 0.5-ml polypropylene tubes and stored at -20 °C until analysis. The minimal time interval between sampling was 15 minutes, and sampling volume did
not exceed 20 µl each time to avoid significant changes of pressure and CSF composition in the subarachnoid space. The total sampling period did not exceed 7 hours.

2.4.3 Microdialysate sample collection in kinetic studies

Sample collection was undertaken two days after microdialysis probe implantation. The stainless steel pins attached to the inlet and outlet polythene tubing of the microdialysis probe were removed. The inlet tubing was attached to a piece of 90-cm long polythene tubing (ID 0.28 mm), which was attached to a 5-ml syringe and needle that was filled with artificial CSF. The outlet tubing was attached to a piece of 60-cm long polythene tubing (ID 0.28 mm). The volume in both the inlet and outlet tubings was treated as dead volume and this was corrected subsequently. During microdialysis, the artificial CSF flow rate was set at 2 µl/minute. Before drug administration, the tubing was flushed with artificial CSF for 30 minutes and the microdialysate used as baseline samples. After IV or IP drug administration (e.g. PHT, FosPHT or TGB), the microdialysate was collected at 10 minutes intervals into 0.5-ml sampling vials and stored at −70 °C until analysis.

2.4.4 Microdialysate sample collection in the study of brain extracellular amino acids

Microdialysis was carried out using the same procedure as in section 2.2.10 except that it was collected at every 15 minutes. Microdialysate collected prior to stimulation was treated as baseline. Microdialysis continued until three hours after electrical stimulation was terminated.
2.5 Phenytoin analysis

PHT concentrations in plasma, CSF and microdialysate were measured by a reverse-phase high performance liquid chromatography (HPLC) system based on a modification of the method of Ratnaraj et al. (1989). A typical PHT chromatogram is shown in Figure 2.4. The lower limit for quantification was 4.9 μmol/L and 0.25 μmol/L for blood and saline samples respectively with coefficient of variation at 7% and 14% respectively. The lower limit of detection was 0.01 μmol/L.

Figure 2.4. A typical PHT chromatogram. There is an early acetonitrile peak, followed by several small, unidentified peaks, and then the PHT peak at 6.08 minutes and the internal standard peak at 6.96 minutes.

2.5.1 High performance liquid chromatography system

This HPLC system comprised of a Spectra-Physics Analytical Spectrasystem (Spectra-Physics, USA) equipped with a P4000 pump, AS1000 autosampler, UV 2000 ultraviolet detector and Chromjet integrator. Chromatograms were run at ambient temperature on a Merck column HI-BAR (250 x 4.0 mm) packed with LiChrospher 100 5 μm (BDH, Poole, U.K.). The column effluent was monitored at
215 nm with a sensitivity range of 0.03 absorption unit full scale. Results were collected using Unipoint Software System (Ver 1.71).

2.5.2 Reagents

Preparation of precipitating reagent (PPR-I) for PHT analysis

PPR-I was made up by mixing 150 µl 10-methoxycarbazepine (internal standard; Sigma-Aldrich, Dorset, UK) with 20 ml acetonitrile (Rathburn Chemicals Limited, Scotland, UK).

Preparation of HPLC mobile phases

Mobile phase line A: phosphate buffer (0.045 M), pH 3, with 15% acetonitrile

12.24 g KH₂PO₄ (Sigma-Aldrich, Dorset, UK) were dissolved in 800 ml HPLC grade water. This solution was pH-adjusted to 3 with orthophosphoric acid (Sigma-Aldrich, Dorset, UK). 300 ml of acetonitrile (Rathburn Chemicals Limited, Scotland, UK) and 900 ml of HPLC grade water were also added into this solution. This was used as the mobile phase for line A.

Mobile phase line B: phosphate buffer (0.0225 M), pH 3, with 37.5% acetonitrile

500 ml of mobile phase line A, 300 ml of acetonitrile and 200 ml of HPLC grade water were mixed together. This was used as the mobile phase for line B. Both mobile phase line A and B were filtered through filtering membrane (pore size: 0.45 µm, Millipore, Ireland) with a vacuum filtering unit before use.
2.5.3 PHT standards and quality control

Preparation of PHT stock-solution and sub-stock solution

100 mg 5,5-diphenyhydantoain (molecular weight 252, Sigma-Aldrich, Dorset, UK) were dissolved in 10 ml HPLC grade methanol (Fisher Scientific International Inc., Leicestershire, UK) to make the concentration of stock-solution to 10 mg/ml.

PHT serum sub-stock solution (concentration of 198 μmol/L) was made up by mixing PHT stock-solution with 20 ml drug free serum (Sigma-Aldrich, Dorset, UK). Saline PHT sub-stock solution was made up by mixing PHT stock-solution with saline. The concentration of saline PHT sub-stock solution for CSF and microdialysate analysis was 39.6 μmol/L and 9.9 μmol/L respectively.

Preparation of PHT standards

PHT standards for serum, CSF and microdialysate analysis were made up by diluting serum or saline sub-stock solution with serum or saline respectively to the concentrations shown in Table 2.1. Calibration of these standards indicated their linearity (Figure 2.5, 2.6 and 2.7; Y-axes in these figures indicated the amount of PHT presented as the area under the PHT peaks in the PHT chromatograms).
Figure 2.5. A typical calibration curve for PHT serum standards.

![Calibration Curve for PHT Serum Standards](image)

Figure 2.6. A typical calibration curve of PHT standards for CSF samples.

![Calibration Curve for PHT CSF Samples](image)

Figure 2.7. A typical calibration curve of PHT standards for microdialysate samples.

![Calibration Curve for PHT Microdialysate Samples](image)
Table 2.1. PHT standards.

<table>
<thead>
<tr>
<th></th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHT concentration in serum standards (µmol/L)</td>
<td>9.9</td>
<td>19.8</td>
<td>39.6</td>
<td>79.2</td>
<td>158.4</td>
<td>-</td>
</tr>
<tr>
<td>PHT concentration in saline standards for CSF analysis (µmol/L)</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>16</td>
<td>32</td>
<td>-</td>
</tr>
<tr>
<td>PHT concentration in saline standards for microdialysate analysis (µmol/L)</td>
<td>0.25</td>
<td>0.5</td>
<td>1</td>
<td>3</td>
<td>5.9</td>
<td>9.9</td>
</tr>
</tbody>
</table>

Preparation of PHT quality control samples and method validation

Quality control (QC) samples in serum and saline were prepared by adding known amount of PHT to serum or saline respectively. QC samples were analysed on numerous occasions so as to test the reproducibility of the analysing method. The within-batch and between-batch imprecision for PHT determination was shown in Table 2.2. Results indicated that the method used to analyse PHT was able to generate reproducible results.

2.5.4 Sample processing for PHT analysis

Blood sample processing

Plasma (50 µl) was pipetted into a 1.5-ml polyethylene tube (Elkay Laboratory Products, UK Ltd) containing 75 µl of PPR-I, vortex mixed and then centrifuged for 5 minutes at 10,000 rpm (Abbott Micro-centrifuge, Abbott, Maidenhead, UK). 75 µl of the supernatant extract were then injected into the HPLC system for analysis.
Table 2.2. Within-batch and between-batch imprecision for the measurement of PHT in serum and saline by HPLC. QC: quality control; SD: standard deviation; CV: coefficient of variation.

<table>
<thead>
<tr>
<th>PHT QC (µmol/L)</th>
<th>Within Batch (n = 10)</th>
<th>Between Batch (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (µmol/L)</td>
<td>SD</td>
</tr>
<tr>
<td>99.0</td>
<td>97.5</td>
<td>0.08</td>
</tr>
<tr>
<td>59.4</td>
<td>59.3</td>
<td>0.02</td>
</tr>
<tr>
<td>19.8</td>
<td>20.1</td>
<td>0.06</td>
</tr>
<tr>
<td>9.9</td>
<td>9.9</td>
<td>0.06</td>
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<tr>
<td>4.0</td>
<td>4.0</td>
<td>0.02</td>
</tr>
<tr>
<td>0.3</td>
<td>0.3</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Blood sample processing for the analysis of plasma free (non-protein bound) PHT

Plasma free (non-protein bound) PHT was prepared by filtering 300 µl plasma (by mixing plasma from two rats) using Centrifree Centrifugal filter devices (Millipore Corporation, Bedford, MA, USA) and spinning at 3500 rpm for 10 minutes. 50 µl plasma filtrate were then mixed with same volume of PPR-I, vortex mixed for 10 seconds, and spun for 10 minutes at 3500 rpm. The supernatant (40 µl) was directly injected into the HPLC system for PHT analysis.

CSF sample processing for PHT analysis

20 µl CSF were mixed with 25 µl PPR-I in a 1.5-ml polyethylene tube and vortex mixed for 10 seconds. 40 µl supernatant were directly injected into the HPLC system.
Microdialysate sample processing for PHT analysis

20 µl of microdialysate were placed directly into HPLC vial, which contained 20 µl PPR-I. After thorough mixing, the mixture was directly injected into the HPLC.

2.6 Tiagabine analysis

TGB in serum, CSF and microdialysate were analysed by HPLC using a method modified by Ratnaraj et al. (2003). A typical TGB chromatogram is shown in Figure 2.8. The lower limit of detection for TGB is 0.1 nmol/L and the lower limit of quantification is 0.5 nmol/L. The coefficient of variation is 6 %.

Figure 2.8. A typical TGB chromatogram. There is an early solvent peak, followed by several small, unidentified peaks, and then TGB peak occurs at just after 10 minutes.

2.6.1 High performance liquid chromatography system

The HPLC system for TGB analysis consisted of: analytical Gilson 307 pump, analytical Gilson 234 autoinjector, ESA coulochem detector, analytical cell model 5011, Hypersil BDS-C18, 3 µm, 125 x 3 mm column (Hewlett Packard,
Stockport, Cheshire), LiChrosphere select B 4 x 4 (5 μm) pre-column (Hewlett Packard, Stockport, Cheshire). Results were collected using Unipoint System Software (Ver 1.71). The electrochemical detector set-up was as follows: Guard cell was set at 950 mV; Electrode 1 was set at 650 mV with current at 50 nAmp; Electrode 2 was set at 850 mV with current at 10 nAmp.

2.6.2 Reagents

Preparation of precipitating reagent (PPR-II) for TGB analysis

5 ml HPLC grade acetonitrile (Fisher Scientific UK Ltd, Leicestershire, UK) for electrochemical detection were dispersed into a scintillation vial.

Preparation of mobile phase

Phosphate buffer A: 14.196 g of Na$_2$HPO$_4$ (BDH-Merck, Leicestershire, UK) were reconstituted in 1000 ml of HPLC grade water (Fisher Scientific UK Ltd, Leicestershire, UK) for electrochemical detection.

Phosphate buffer B: 13.809 g of KH$_2$PO$_4$ (BDH-Merck, Leicestershire, UK) were reconstituted in 1000 ml of HPLC grade water for electrochemical detection.

Preparation of phosphate buffer (0.1 M), pH 8.04, with 35% methanol and 15% acetonitrile

60 ml of phosphate buffer A, 40 ml of phosphate buffer B, 30 ml of acetonitrile and 70 ml of methanol were mixed and filtered using the Millipore filtration unit (Millipore, Ireland). 0.114 g of 1-octanesulphonic acid (BDH-Merck, Leicestershire, UK) were subsequently dissolved in the filtered buffer.
2.6.3 TGB Standards and quality control

Preparation of TGB stock-solution

0.412 mg of TGB hydrochloride was dissolved in 100 ml HPLC grade methanol (Fisher Scientific UK Ltd, Leicestershire, UK) to make up to 10 μmol/L.

Preparation of TGB standards

TGB serum standards were made up to concentrations shown in Table 2.3 using drug-free human sera (SCIPAC Ltd, Sittingbourne, Kent, UK) and TGB stock solution. TGB standards for CSF and microdialysate analysis were made up to concentrations shown in Table 2.3 using saline and TGB stock solution. Calibration of these standards indicated their linearity (Figure 2.9, 2.10 and 2.11; Y-axes in these figures indicated the amount of TGB presented as the area under the TGB peaks in the TGB chromatograms).

Table 2.3. TGB standards.

<table>
<thead>
<tr>
<th></th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
</tr>
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<tbody>
<tr>
<td>Serum standards (nmol/L)</td>
<td>25</td>
<td>50</td>
<td>100</td>
<td>200</td>
<td>400</td>
<td>800</td>
</tr>
<tr>
<td>Standards for CSF (nmol/L)</td>
<td>5</td>
<td>10</td>
<td>20</td>
<td>40</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Standards for microdialysate (nmol/L)</td>
<td>2</td>
<td>5</td>
<td>10</td>
<td>20</td>
<td>40</td>
<td>-</td>
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</table>

Preparation of TGB QC and method validation

TGB QC in serum and saline were made up by adding known amount of TGB to serum and saline respectively. QC samples were run for a number of times to test the reproducibility of the analysing method. The within-batch and between-batch imprecision for TGB measurement are shown in Table 2.4. Results indicated that the
Figure 2.9. A typical calibration curve for TGB serum standards.

Figure 2.10. A typical calibration curve of TGB standards for CSF samples.

Figure 2.11. A typical calibration curve of TGB standards for microdialysate samples.
method used to analyse TGB was able to generate reproducible results.

Table 2.4. Within-batch and between-batch imprecision for the measurement of TGB in serum and saline by HPLC. QC: quality control; SD: standard deviation; CV: coefficient of variation.

<table>
<thead>
<tr>
<th>TGB QC (nmol/L)</th>
<th>Within-batch (n = 18)</th>
<th>Between-batch (n = 30)</th>
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<tr>
<td></td>
<td>Mean (nmol/L)</td>
<td>SD</td>
</tr>
<tr>
<td>700</td>
<td>696.0</td>
<td>7.59</td>
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<td>300</td>
<td>296.3</td>
<td>6.78</td>
</tr>
<tr>
<td>30</td>
<td>30.3</td>
<td>1.34</td>
</tr>
<tr>
<td>12</td>
<td>11.9</td>
<td>0.04</td>
</tr>
<tr>
<td>6</td>
<td>6.1</td>
<td>0.02</td>
</tr>
<tr>
<td>3</td>
<td>3.2</td>
<td>0.03</td>
</tr>
</tbody>
</table>

2.6.4 Sample processing for TGB analysis

**Blood sample processing**

50 μl of serum standard or diluted rat serum were pipetted into a 1.5-ml polyethylene tube (Elkay Laboratory Products, UK Ltd) containing 100 μl of PPR-II, vortex mixed for 10 seconds and then centrifuged for 5 minutes at 10,800 rpm (Abbott Micro-centrifuge, Abott, Maidenhead, U.K.). 100 μl of the supernatant extract were transferred into the HPLC system (injection volume was 5 μl).

**CSF and microdialysate sample processing**

20 μl of animal CSF or saline CSF standard were pipetted into a 1.5 ml polyethylene tube containing 25 μl of PPR-II, vortex mixed for 10 seconds and then
centrifuged for 5 minutes at 10,800 rpm. 40 μl of the supernatant extract were transferred into the HPLC system (injection volume was 5 μl). Microdialysate samples were processed exactly the same except that the injection volume was 10 μl.

2.7 Analysis of brain amino acids

An automated precolumn derivatisation method for the measurement of amino acids in the brain ECF microdialysate was used, adapted from the method described by Shah et al. (1999). It involves labelling amino acids with naphthalene-2,3-dicarboxaldehyde in the presence of cyanide (CN'). The resulting highly stable N-substituted 1-cyanobenz[f]isoindole derivatives were separated using a gradient system and detected fluorometrically.

2.7.1 High performance liquid chromatography system

This HPLC system comprised of an injector of Shimadzu model SIL-10AD VP with 50 μl loop, a pump of Shimadzu model LC-10AT VP, Perkin Elmer Series 200 Fluorescence Detector. Chromatograms were run at ambient temperature on Beckman column of ultrasphere ODS 5 μm, 25 cm x 4.6 mm (id). The column effluent was monitored at excitation wavelength of 442 nm and emission wavelength of 480 nm.

2.7.2 Reagents

All the chemicals and solvent were obtained from Sigma-Aldrich, UK.

*Mobile Phase*

*Eluant A: Ammonium Acetate buffer 50 mM; pH 7 / Tetrahydrofuran*
3.854 g ammonium acetate were dissolved in 1 litre deionised water and adjusted to pH 7 using 0.1 M NaOH to give a 50 mM solution. 950 ml ammonium acetate buffer was taken and 50 ml tetrahydrofuran was added, filter and degassed.

**Eluant B: Ammonium Acetate buffer 50 mM; pH 7 / Acetonitrile / Methanol**

550 ml acetonitrile and 100 ml methanol were added to 350 ml ammonium acetate buffer. The mixture was filtered and degassed.

**Borate buffer 0.1M; pH 9.5**

61.83 mg sodium borate were dissolved in 9ml distilled water, adjusted to pH 9.5 with 0.1 M NaOH. The solution was then made up to 10 ml with distilled water and stored in an amber bottle at 4°C.

**Potassium cyanide 10mM**

6.5 mg potassium cyanide were dissolved in 10 ml deionised water and were stored in an amber bottle at 4°C.

**Naphthalene-2,3-dicarboxaldehyde, 3mM**

0.552 mg naphthalene-2,3-dicarboxaldehyde were dissolved in 1 ml methanol and stored in an amber vial at 4°C.

### 2.7.3 Amino acid standards and quality control

Stock solutions of 10 mM of each of glutamate, glutamine, glycine, alanine, taurine and GABA (all obtained from Sigma) were prepared in the water.
A stock mixture of 10 μM was made by adding 100 μl of each into a total of 100 ml in water. The stock mixture was aliquoted into 1-ml lots (in 1.5-ml polypropylene tubes) and stored at -20°C. On the day of analyses an aliquot was diluted as described below. No internal standard was used for this assay. A standard curve was prepared by diluting the stock mixture with borate buffer to give concentrations of 5, 2.5, 1.0 and 0.5 μM.

Table 2.5. Amino acid standards.

<table>
<thead>
<tr>
<th>Concentration (μM)</th>
<th>Volume of Stock Standard (10μM) in μL</th>
<th>Volume of Borate Buffer in μL</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>2.5</td>
<td>250</td>
<td>750</td>
</tr>
<tr>
<td>1.0</td>
<td>100</td>
<td>900</td>
</tr>
<tr>
<td>0.5</td>
<td>50</td>
<td>950</td>
</tr>
<tr>
<td>0.0</td>
<td>0</td>
<td>1000</td>
</tr>
</tbody>
</table>

2.7.4 Sample processing for amino acid analysis

10 μl microdialysate or standard were pipetted into an HPLC vial and 5 μl potassium cyanide, 16 μl borate buffer and 16 μl naphthalene-2,3-dicarboxaldehyde were added. After mixing well samples were injected into HPLC.

2.8 Establishment of self-sustained status epilepticus model

The SSSE model used was based on the model described by Walker et al. (1999).
2.8.1 Material

Stimulating and recording electrodes were constructed as described in section 2.2.4. Other equipment used in this study comprised the stimulator and recording device.

Stimulator

Constant current stimuli were delivered from a Neurolog stimulator (NL304 period generator, NL505 flip-flop, NL800 stimulus isolators: Digitimer Ltd, Welwyn Garden City, UK) as shown in Figure 2.13. The pulse generator was gated so that the pulses could be synchronised with the computer recording. This gating also enabled fixed length trains to be generated, and using a purpose built series of flip-flop circuits, it was possible to generate fixed length trains that occurred at fixed intervals.

Recording device

Potentials were amplified and filtered (0.1 Hz to 5 kHz band pass) via a Neurolog amplifier (Figure 2.12, NL 100A pre-amp, NL 104A AC amplifier, NL 125 filter: Digitimer Ltd, Welwyn Garden City, UK) onto a storage oscilloscope and then via an NB-MIO-16 interface (National Instruments, Berkshire, UK) into computer.
Figure 2.12. Illustration of the amplifier. The amplifier was a differential AC amplifier with B connected to earth, and a built-in high pass filter set at 0.1 Hz. A low pass filter set at 5 kHz was also necessary to reduce high frequency interference.

Figure 2.13. Illustration of the stimulator. The pulse width could be set at 50, 150 or 500 μs; the frequency of pulses could be set from 0-10kHz; the amplitude could be set from 0-10mA, and using the alternating switch the polarity of the pulses from the constant current generators could be altered.
2.8.2 Induction of self-sustained status epilepticus

Seven to 10 days after electrode implantation, the location of the stimulation electrodes was tested by stimulating the perforant path (angular bundle) and observing the population spikes in freely moving animals. Rats in which population spikes were more than 1 mV in amplitude and could be elicited at a current level of 2 mA or less were used in the experiments. The perforant path was stimulated using Walker's protocol (Walker et al. 1999) with continuous 4 mA, 50 µs monopolar pulses at 20 Hz for 2 hours to induce SSSE. Potentials were amplified and filtered (0.1-500 Hz band pass) via a Neurolog amplifier (Digitimer, Welwyn Garden City, UK) onto a storage oscilloscope and then via an analogue to a digital interface onto a computer running Labview (National Instruments, Berkshire, UK; sample rate 1kHz). During and, in some animals, after stimulation, Racine stage 5 (Racine, 1972) (Table 2.6) seizures were sometimes observed. Approximately 30-50 minutes after the beginning of stimulation, regular, large-amplitude spontaneous discharges independent of the electrical stimulation occurred in over 90% of the animals; the experiment was terminated in animals in which such discharges did not occur (which indicated that the SSSE would not be established in that particular animal).

After the end of 120-minute stimulation, regular spikes at 1Hz and above and seizures ranging from Racine stage 1-5 (Racine et al., 1972) continued for at least 180 minutes until proper interventions were applied (test drugs or propofol).
Table 2.6. Status epilepticus-motor seizure classification (Racine et al., 1972).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Behaviour Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mouth and facial movements</td>
</tr>
<tr>
<td>2</td>
<td>Head nodding</td>
</tr>
<tr>
<td>3</td>
<td>Forelimb clonus</td>
</tr>
<tr>
<td>4</td>
<td>Rearing</td>
</tr>
<tr>
<td>5</td>
<td>Rearing and falling (including a full motor seizure, with loss of postural control)</td>
</tr>
</tbody>
</table>

2.9 Histology procedures

Unless otherwise stated, all the chemicals and solution were purchased from Sigma-Aldrich (Dorset, UK).

2.9.1 Preparation of 4% paraformaldehyde solution

Salt-solution: di-sodium hydrogenphosphate-dihydrate (8.15 g) and sodium-dihydrogenphosphate-monohydrate (4 g) were dissolved in 300 ml distilled water at room temperature. Paraformaldehyde (40 g) powder was added to 700 ml hot distilled water (65 °C). The solution was stirred until the paraformaldehyde powder was completely dissolved. Subsequently the solution was adjusted to pH 7.35 using sodium hydroxide (1M). It was then mixed with the salt solution and stored at 4 °C.

2.9.2 Heart perfusion

Rats were deeply anaesthetised with IP pentobarbitone, placed on their back and their four limbs were fixed on a perfusion table with adhesive tape. The
chest and rib cage were opened from the left margin of the sterna and heart exposed. A 13-G needle attached to a cannula filled with saline was advanced into the left ventricle. Another incision was made in the right auricle. The saline then was allowed to flush into the left ventricle at a pressure of 1-2 bar until the animal had been exsanguinated. The saline was then replaced by freshly made 4% paraformaldehyde solution and the perfusion continued into the left ventricle at a pressure of 1-3 bar for 10-15 minutes. When rats exhibited involuntary twitching in their four limbs, which indicated good perfusion all through the body, the perfusion was terminated and all cannula and needles removed.

### 2.9.3 Brain separation and fixation

After the heart perfusion, rats were turned upside-down to rest on their chest. A 2.5-cm incision was made on the skin at the top of the head. The skull was opened carefully from the midline and the occipital crest. The whole brain was carefully freed from the base, the optical chiasm and the spinal cord using a fine spatula. The whole brain was carefully transferred to a falcon tube containing 4% paraformaldehyde solution and then kept at 4 °C for 2-3 weeks prior to processing.

### 2.9.4 Processing the brains

The rat brains were processed in a Shandon Citadel 2000 Tissue Processor (Pacific Southwest Lab Equipment Inc, CA, USA) for three days according to the protocol shown in Table 2.7. After processing, the rat brains were embedded in paraffin.
Table 2.7. Processing protocol for rat brains. Rat brains were processed in each of the reagents sequentially to be dehydrated, cleared of ethanol and infiltrated with paraffin wax. This protocol was carried out automatically in the tissue processor.

<table>
<thead>
<tr>
<th>Step</th>
<th>Reagent</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70% Ethanol</td>
<td>6 hours</td>
</tr>
<tr>
<td>2</td>
<td>90% Ethanol</td>
<td>6 hours</td>
</tr>
<tr>
<td>3</td>
<td>Absolute Ethanol</td>
<td>30 hours</td>
</tr>
<tr>
<td>4</td>
<td>Chloroform</td>
<td>12 hours</td>
</tr>
<tr>
<td>5</td>
<td>Wax</td>
<td>18 hours</td>
</tr>
</tbody>
</table>

2.9.5 Sectioning and staining

Coronal sections (20 μm) were taken using a Leitz sledge microtome throughout the rat brain. Slides were dewaxed in xylene, placed in alcohol, washed, stained with cresyl fast violet for 20 minutes at 60 °C and then differentiated in 95% alcohol. Cresyl fast violet stains Nissl granules and cell nuclei through its binding with anionic nucleic acids. Neurons were distinguished from other cell types on the basis of their size, the presence of Nissl granules in the cytoplasm, and a large pale staining nucleus with one or more nucleoli.

2.9.6 Assessment of neuronal damage

Neuronal damage was assessed by neuronal loss in CA1, CA3 and hilus from either side of the hemisphere. An optical dissector microscope (Zeiss) with digital length gauge (Heidenhaim, Illinois) was used. Neuron counting was performed in two slides (220 μm apart, from either side of the recording electrode) in
each rat. In each slide ten boxes in CA1, CA3 and hilus respectively were used to count the neurons (Figure 2.14). Neurons in three-dimensional boxes were counted in the cell body layer of the CA1, hilus, and CA3 according to the rules described by Williams & Rakic (1988): Cell nuclei completely inside the box were counted; Cell nuclei completely outside the box were not counted; Cell nuclei that touch the bottom, front and left planes of the counting box were not counted; Cell nuclei that touch top, back or right planes were counted. A mean value of the neuronal density in the 3-dimensional box (20 μm x 20 μm x 5 μm) was acquired for the CA1, CA3 and hilus regions respectively. Both hemispheres were assessed and compared. The analysis was undertaken in a blinded fashion. The observer was unaware of the treatment regimen.

2.10 Pharmacokinetic analysis

Pharmacokinetic parameters were calculated in Microsoft Excel programme (Version 97) using equations based on a one-compartment or two-compartment model (where appropriate) with first order elimination. It was assumed that the bioavailability of drugs given IV and IP was 100%.

Where a two-compartment model was applied, it was assumed that data could be modelled using equation $C(t) = Ae^{-\alpha t} + Be^{-\beta t}$; $B$ and $\beta$ could be approximated for the log-linear regression analysis for a single exponential function of the terminal concentrations; $\alpha$ was calculated from the log-linear regression analysis for a single exponential function of $C(t) - Be^{-\beta t}$ (i.e. by curve stripping).
Figure 2.14. Illustration of areas for neuron counting in the coronal section of the hippocampus from an unstimulated rat. Neuron counting was performed at the cell body layer in the middle of the CA1 and CA3 regions and at the start of hilus region (indicated by the red boxes in the photograph). Ten continuous boxes (20 μm x 20 μm x 5 μm each) were counted with boxes moving in a step-wise manner.
Tmax, Cmax, elimination rate constant (Ke), $t^{1/2}$, AUC values were determined. AUC values were calculated based on conventional linear trapezoid summation and extrapolation techniques to extrapolate to infinite time as appropriate. The Ke was estimated by least-square regression analysis of the terminal log-linear portion of the concentration versus time profile. The $t^{1/2}$ was calculated by dividing the natural log of 2 by Ke. Cmax and Tmax were read directly from the concentration versus time curves. Ratios of CSF concentration to serum concentration, and ECF concentration to serum concentration were calculated for each individual animal at each time point for which there were concurrent data.

2.11 Statistical analysis

The non-parametric Mann-Whitney U test was used to study the significance of results. A P value of 0.05 was regarded as statistically significant.
3 PHARMACOKINETICS AND NEUROPHARMACOKINETICS OF PHENYTOIN AFTER PHENYTOIN AND FOSPHENYTOIN ADMINISTRATION

3.1 Introduction

Because of the extensive use of PHT in the management of epilepsy in general and its use specifically in SE, along with the fact that it has some unique and peculiar pharmacokinetic characteristics, the central (CSF and ECF) and peripheral (blood) kinetic inter-relationship of PHT has been extensively studied in both man and in animals (Walker et al., 1996; Lolin et al., 1994; Scheyer et al., 1994a; Ogiso et al., 1993; Baron et al., 1983). In contrast, kinetic studies of FosPHT have been confined to the blood compartment, primarily in man, although one animal study reported on its blood kinetics (Loscher et al., 1998) whilst a second study reported on its blood and whole brain kinetics (Walton et al., 1999). In man, PHT and FosPHT appear to be bio-equivalent and the conversion half-life of FosPHT to PHT in healthy male adults is approximately 9 minutes (Jamerson et al., 1990). FosPHT is extensively bound (95%) to blood albumin and consequently displaces PHT (which is 90% bound) resulting in elevated free PHT concentrations (Lai et al., 1995). Typically, PHT binding returns to normal as plasma FosPHT concentrations decline (30-60 minutes post FosPHT dose).

With the exception of a single study in the rats (Walton et al., 1999), whereby whole brain concentrations of PHT were measured after IV PHT and FosPHT, the inter-relationship between central and peripheral kinetics has not been investigated. That whole brain PHT concentrations were lower after FosPHT administration compared to that after equal molar of PHT administration is
interesting. However, whole brain drug concentrations do not necessarily reflect drug content at the site of drug action, as these represent the mixed concentrations of the unbound drug in the intracellular space, ECF and that binds to the receptors on the cell membrane, as well as those inside the cerebral blood vessels (both bound and unbound). As only the drug that is present in the ECF will be able to exert a pharmacological effect, the measurement of ECF drug content is more appropriate. In this study the inter-relationship of PHT pharmacokinetics in blood (plasma), neuropharmacokinetics in CSF and brain ECF (frontal cortex and hippocampus) were investigated after IV administration of PHT and FosPHT in the freely behaving rat.

3.2 Methods

Males Sprague-Dawley rats weighing 250-350 g were used in all the experiments. Rats were allowed at least 48 hours to get acclimatised in the animal laboratory before experiments.

Blood, CSF catheters and microdialysis probes were constructed as described in Section 2.2.1 through Section 2.2.3 and subsequently in vitro recovery for microdialysis probes carried out as described in Section 2.3.3. The probe recovery rate was 18 ± 2 % and was used to adjust the PHT ECF data. Either blood and CSF catheters or blood and microdialysis probes were implanted accordingly as described in Section 2.3.

Two days after the implantation of the catheters and probes, rats were chosen randomly to receive IV infusion of either PHT or FosPHT, at either 30 mg/kg or 60 mg/kg for rats taken blood and CSF samples and at 60 mg/kg for rats taken blood and microdialysate samples. FosPHT doses presented in this thesis were all
PE. PHT was infused at 100 μl/minute while FosPHT was infused at 200 μl/minute. Blood, CSF and microdialysate samples were collected as described in Section 2.4.1 through 2.4.3.

Total and free (non-protein bound) PHT contents in blood and PHT content in CSF and microdialysate were analysed as described in Section 2.5.

Pharmacokinetic analysis was carried out using the Microsoft Excel programme. First order elimination was assumed and the two-compartment model was used for blood samples and the one-compartment model used for CSF and microdialysate samples as described in Section 2.10. Tmax, Cmax, AUC and t½ were computed for PHT in plasma, CSF and microdialysate. Statistical analysis was carried out using Mann-Whitney U test as described in Section 2.11.

3.3 Pharmacokinetics and neuropharmacokinetics of phenytoin after phenytoin administration

3.3.1 Pharmacokinetics of phenytoin in the blood compartment

The pharmacokinetic parameters after PHT administration at 30 mg/kg and 60 mg/kg are shown in Tables 3.1 and 3.2. PHT Cmax was achieved at the end of IV infusion of PHT. During the subsequent 10 minutes, concentrations rapidly declined, and the decline continued in an exponential manner (Figure 3.1). The distribution half-life was 9.5 ± 0.4 minutes and 9.7 ± 0.7 minutes in 30 mg/kg and 60 mg/kg group respectively (P > 0.05, Mann-Whitney U test). PHT Cmax values increased dose-dependently. AUC and t½ values increased disproportionately to dose. Dose-adjusted AUC was statistically different between the two dose groups (P < 0.05, Mann-Whitney U test). Plasma t½ was also significantly different in these
two dose groups (P < 0.01, Mann-Whitney U test). Thus doubling the dose of PHT was associated with a three-fold increase in t½ values.

Free PHT concentrations were determined by pooling samples from two rats. Samples used comprised of those collected between 5 and 60 minutes after PHT administration. The free / total PHT concentration ratio in plasma was 0.268 ± 0.001 for the 30 mg/kg PHT group and 0.257 ± 0.003 for the 60 mg/kg PHT group (Figure 3.2). There is no difference between 30mg/kg PHT and 60 mg/kg PHT administered groups (P > 0.05, Mann-Whitney U test).

Table 3.1. Pharmacokinetic parameters of PHT in plasma after 30mg/kg PHT administration.

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Cmax (μmol/L)</th>
<th>AUC (μmol h/L)</th>
<th>t1/2 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>81</td>
<td>88</td>
<td>34</td>
</tr>
<tr>
<td>2</td>
<td>214</td>
<td>149</td>
<td>41</td>
</tr>
<tr>
<td>3</td>
<td>142</td>
<td>85</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>191</td>
<td>127</td>
<td>59</td>
</tr>
<tr>
<td>5</td>
<td>149</td>
<td>109</td>
<td>61</td>
</tr>
<tr>
<td>6</td>
<td>167</td>
<td>117</td>
<td>56</td>
</tr>
<tr>
<td>Mean</td>
<td>157</td>
<td>112</td>
<td>46</td>
</tr>
<tr>
<td>± SEM</td>
<td>19</td>
<td>10</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 3.2. Pharmacokinetic parameters of PHT in plasma after 60mg/kg PHT administration.

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Cmax (μmol/L)</th>
<th>AUC (μmol h/L)</th>
<th>t1/2 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>426</td>
<td>621</td>
<td>118</td>
</tr>
<tr>
<td>2</td>
<td>340</td>
<td>731</td>
<td>165</td>
</tr>
<tr>
<td>3</td>
<td>349</td>
<td>658</td>
<td>169</td>
</tr>
<tr>
<td>4</td>
<td>290</td>
<td>282</td>
<td>84</td>
</tr>
<tr>
<td>5</td>
<td>283</td>
<td>388</td>
<td>113</td>
</tr>
<tr>
<td>Mean</td>
<td>338</td>
<td>536</td>
<td>130</td>
</tr>
<tr>
<td>± SEM</td>
<td>26</td>
<td>86</td>
<td>16</td>
</tr>
</tbody>
</table>
Figure 3.1. Plasma total PHT concentration versus time profiles after 30 mg/kg and 60 mg/kg PHT administration. Values are mean ± SEM (n = 5-6).

Figure 3.2. Plasma free / total PHT concentration ratios after 30 mg/kg and 60 mg/kg PHT administration. Values are mean ± SEM (n = 5).
3.3.2 Neuropharmacokinetics of phenytoin in the cerebrospinal fluid compartment

The neuropharmacokinetic parameters of PHT in CSF after 30 mg/kg and 60 mg/kg PHT administration are shown in Tables 3.3 and Table 3.4 respectively. PHT readily penetrated into the CSF compartment and was detectable at the time of first CSF sampling (5 minutes postdose). Cmax increased dose-dependently, and subsequently PHT concentration declined exponentially (Figure 3.3). Tmax was 9 ± 1 minutes for the 30 mg/kg PHT administered group and 13 ± 4 minutes for the 60 mg/kg PHT administered group. However the difference of Tmax between the two doses was not statistically significant (P > 0.05, Mann-Whitney U test). AUC and t½ values increased disproportionately to dose. t½ and dose-adjusted AUC were significantly different between the 30 mg/kg PHT group and the 60 mg/kg PHT group (P < 0.01, Mann-Whitney U test).

Equilibration between the CSF and blood compartments occurred by 15 minutes after PHT administration and was dose-independent. The CSF to plasma PHT concentration ratio was 0.168 ± 0.008 for the 30 mg/kg PHT group and 0.193 ± 0.006 for the 60 mg/kg PHT group (Figure 3.4). Values at each time point were not different in these two dose groups (P > 0.05, Mann-Whitney U test).

Comparison between plasma PHT t½ value and CSF PHT t½ value revealed that there was no statistically significant difference between them for both 30 mg/kg and 60 mg/kg PHT groups (P > 0.05, Mann-Whitney U test).
Table 3.3. Neuropharmacokinetic parameters of PHT in CSF after 30mg/kg PHT administration.

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Tmax (min)</th>
<th>Cmax (μmol/L)</th>
<th>AUC (μmol h/L)</th>
<th>t1/2 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>15</td>
<td>16</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
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<td>11</td>
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</tr>
<tr>
<td>5</td>
<td>8</td>
<td>19</td>
<td>31</td>
<td>72</td>
</tr>
<tr>
<td>Mean</td>
<td>9</td>
<td>15</td>
<td>23</td>
<td>57</td>
</tr>
<tr>
<td>± SEM</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 3.4. Neuropharmacokinetic parameters of PHT in CSF after 60mg/kg PHT administration.

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Tmax (min)</th>
<th>Cmax (μmol/L)</th>
<th>AUC (μmol h/L)</th>
<th>t1/2 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>25</td>
<td>72</td>
<td>132</td>
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<tr>
<td>2</td>
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<td>Mean</td>
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<tr>
<td>± SEM</td>
<td>4</td>
<td>5</td>
<td>14</td>
<td>25</td>
</tr>
</tbody>
</table>
Figure 3.3. PHT concentration versus time profiles in CSF after 30mg/kg and 60 mg/kg PHT administration. Values are mean ± SEM (n = 5-6).

Figure 3.4. CSF / plasma PHT concentration ratios after 30 mg/kg and 60 mg/kg PHT administration. Values are mean ± SEM (n = 5-6).
3.3.3 Neuropharmacokinetics of phenytoin in brain extracellular fluid compartment

The neuropharmacokinetic parameters of PHT in brain ECF from frontal cortex and hippocampus are shown in Tables 3.5 and 3.6. After IV administration at 60 mg/kg, PHT penetrated the BBB readily. PHT was detectable at the time of first dialysate sampling (10 minutes). But the Cmax value in the frontal cortex and the hippocampus were not reached until approximately half an hour later (34 ± 3 and 30 ± 5 minutes respectively). Concentrations subsequently declined exponentially. There was a small second PHT concentration peak at 210-240 minute postdose in both the frontal cortex and hippocampus (Figure 3.5). The frontal cortex AUC and $t_{1/2}$ values were comparable to those in the hippocampus ($P > 0.05$, Mann-Whitney U test).

Equilibration between brain ECF and plasma was reached by 30 minutes postdose. The ratio of PHT concentration in brain ECF to that in plasma was 0.062 ± 0.0018 and 0.059 ± 0.0011 for frontal cortex and hippocampus respectively (Figure 3.6). Values at each time point were not different between these two regions ($P > 0.05$, Mann-Whitney U test).

Comparison between plasma and brain ECF compartments revealed that the $t_{1/2}$ in brain frontal cortex and hippocampal ECF were not different from that in plasma ($P > 0.05$, Mann-Whitney U test).
Table 3.5. Neuropharmacokinetic parameters of PHT in frontal cortex ECF after 60 mg/kg PHT administration.

<table>
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<tr>
<th>Rat No</th>
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</tbody>
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Table 3.6. Neuropharmacokinetic parameters of PHT in hippocampal ECF after 60 mg/kg PHT administration.

<table>
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<tr>
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<th>AUC (μmol h/L)</th>
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Figure 3.5. PHT concentration versus time profiles in frontal cortex and hippocampal ECF after 60 mg/kg PHT administration. Values are mean ± SEM (n = 7-8).

Figure 3.6. ECF / plasma PHT concentration ratios in frontal cortex and hippocampus after 60 mg/kg PHT administration. Values are mean ± SEM (n = 7-8).
3.4 Pharmacokinetics and neuropharmacokinetics of phenytoin after fosphenytoin administration

3.4.1 Pharmacokinetics of PHT in blood compartment

The pharmacokinetic parameters of PHT after FosPHT administration at 30 mg/kg and 60 mg/kg are shown in Tables 3.7 and 3.8 respectively. PHT Cmax was achieved by the end of IV infusion of FosPHT. During the subsequent 10 minutes, concentrations rapidly declined, and the decline continued in an exponential manner (Figure 3.7). The distribution half-life of PHT was $11.8 \pm 1.4$ minutes and $15.7 \pm 2.4$ minutes in 30 mg/kg and 60 mg/kg group respectively ($P > 0.05$, Mann-Whitney U test). PHT Cmax values increased dose-dependently after administration of FosPHT. Dose-adjusted Cmax in the 30 mg/kg group was not different from that in the 60 mg/kg group ($P > 0.05$, Mann-Whitney U test). AUC values and $t\frac{1}{2}$ values increased disproportionately to dose ($P < 0.05$, Mann-Whitney U test).

Table 3.7. Pharmacokinetic parameters of PHT in plasma after 30mg/kg FosPHT administration.

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Cmax (μmol/L)</th>
<th>AUC (μmol h/L)</th>
<th>$t\frac{1}{2}$ (min)</th>
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<tr>
<td>± SEM</td>
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<td>17</td>
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</tr>
</tbody>
</table>
Table 3.8. Pharmacokinetic parameters of PHT in plasma after 60mg/kg FosPHT administration.

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Cmax (μmol/L)</th>
<th>AUC (μmol h/L)</th>
<th>t1/2 (min)</th>
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</table>

Figure 3.7. Plasma total PHT concentration versus time profiles after 30 mg/kg and 60 mg/kg FosPHT administration. Values are mean ± SEM (n = 5-7).

Free PHT concentrations were determined by pooling samples from two rats. Samples used comprised those collected 5-60 minutes after FosPHT.
administration. The free / total PHT concentration ratio in plasma was 0.252 ± 0.001 for the 30 mg/kg FosPHT group and 0.259 ± 0.002 for the 60 mg/kg FosPHT group (Figure 3.2). There is no difference between 30mg/kg FosPHT and 60 mg/kg FosPHT administered groups (P > 0.05, Mann-Whitney U test).

Figure 3.8. Free / total PHT concentration ratios in plasma after 30 mg/kg and 60 mg/kg FosPHT administration. Values are mean ± SEM (n = 3).

3.4.2 Neuropharmacokinetics of phenytoin in the cerebrospinal fluid compartment

The CSF PHT concentration versus time profiles after FosPHT administration at 30 mg/kg and 60 mg/kg are shown in Figure 3.9. The corresponding neuropharmacokinetic parameters of PHT in CSF after FosPHT administration at 30 mg/kg and 60 mg/kg are shown in Tables 3.9 and 3.10 respectively.
After administration of FosPHT at either 30 mg/kg or 60 mg/kg, Cmax was achieved rapidly and dose-dependently. The dose-adjusted Cmax values in 30 mg/kg group were not different from that of the 60 mg/kg group (P > 0.05, Mann-Whitney U test). Similarly Tmax values after administration of 30 mg/kg (12 ± 2 minutes) or 60 mg/kg (10 ± 1 minutes) FosPHT were not significantly different (P > 0.05, Mann-Whitney U test). AUC values increased disproportionately to dose. Dose-adjusted AUC values for the 60 mg/kg group was significantly higher than that of the 30 mg/kg FosPHT group (P < 0.01, Mann-Whitney U test) and t½ increased dose-dependently.

By 15 minutes postdose equilibration between CSF and plasma PHT concentrations occurred (Figure 3.10). The concurrent CSF to plasma PHT concentration ratios for the 60 mg/kg FosPHT group were not different from that of 30 mg/kg FosPHT group when the values at each time point were compared (P > 0.05, Mann-Whitney U test).

Comparison between plasma PHT t½ and CSF PHT t½ values revealed that there was no difference between them (P > 0.05, Mann-Whitney U test).

Table 3.9. Neuropharmacokinetic parameters of PHT in CSF after 30 mg/kg FosPHT administration.

<table>
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Table 3.10. Neuropharmacokinetic parameters of PHT in CSF after 60 mg/kg FosPHT administration.

<table>
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<td>Mean ± SEM</td>
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Figure 3.9. PHT concentration versus time profiles in CSF after 30 mg/kg and 60 mg/kg FosPHT administration. Values are mean ± SEM (n = 5-6).
3.4.3 Neuropharmacokinetics of phenytoin in brain extracellular fluid compartment

The pharmacokinetic parameters of PHT in brain ECF from frontal cortex and hippocampus after 60 mg/kg FosPHT administration are shown in Table 3.11 and Table 3.12. After FosPHT administration, PHT penetrated the BBB readily and was detectable at the time of first dialysate sampling (10 minutes). Peak PHT concentrations in frontal cortex and hippocampus were reached by 29 ± 4 and 34 ± 3 minutes postdose respectively. Concentrations subsequently declined exponentially (Figure 3.11). They were not different in frontal cortex and hippocampus at each time point (P > 0.05, Mann-Whitney U test). AUC and t½ values in the frontal cortex were comparable to those in the hippocampus with no statistically significant difference apparent (P > 0.05, Mann-Whitney U test).
Equilibration between brain ECF and plasma was reached by 30 minutes postdose. The ratio of PHT concentration in brain ECF to that in plasma was 0.076 ± 0.0036 and 0.064 ± 0.0027 for frontal cortex and hippocampus respectively (Figure 3.12), and indeed, no statistically significant difference was found between the two brain regions (P > 0.05, Mann-Whitney U test).

Comparison between plasma and brain frontal cortex and hippocampal ECF compartments revealed no statistically significant difference of t½ after 60 mg/kg FosPHT administration (P > 0.05, Mann-Whitney U test).

Figure 3.11. PHT concentration versus time profiles in frontal cortex and hippocampal ECF after 60 mg/kg FosPHT administration. Values are mean ± SEM (n = 6).
Table 3.11. Pharmacokinetic parameters of PHT in frontal cortex ECF after 60 mg/kg FosPHT administration. Values are mean ± SEM.

<table>
<thead>
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<th>AUC (µmol h/L)</th>
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Table 3.12. Pharmacokinetic parameters of PHT in hippocampal ECF after 60 mg/kg FosPHT administration.

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Figure 3.12. ECF / plasma PHT concentration ratios in frontal cortex and hippocampus after 60 mg/kg FosPHT administration. Values are mean ± SEM (n = 6).

3.5 Pharmacokinetic and neuropharmacokinetic comparison of phenytoin after phenytoin and fosphenytoin administration

3.5.1 Pharmacokinetic comparison of phenytoin in blood compartment after phenytoin and fosphenytoin administration

After IV administration of PHT and FosPHT at 30 mg/kg or 60 mg/kg, PHT Cmax values were achieved by the end of IV infusion for both drugs. PHT concentration declined significantly in the first 10 minutes, and then continued to decline exponentially (Figure 3.13). At time 90 minutes and 120 minutes, the PHT concentrations were higher after 60 mg/kg FosPHT than that after 60 mg/kg PHT administration (P < 0.05, Mann-Whitney U test). However, the PHT AUC values were not significantly different (P > 0.05, Mann-Whitney U test). PHT Cmax
increased dose-dependently and values were equal after PHT and FosPHT administration (P > 0.05, Mann-Whitney U test) (Figure 3.14). For both PHT and FosPHT, PHT AUC values and t½ values increased disproportionately to dose. Furthermore, no difference in AUC and t½ values was observed between PHT and FosPHT administrated groups (Figure 3.15 and 3.16), regardless of dose administered (P > 0.05, Mann-Whitney U test). Therefore, overall the pharmacokinetic parameters of PHT in the blood compartment after PHT and FosPHT IV administration were indistinguishable.

The plasma free / total PHT concentration ratio after PHT and FosPHT administration at 30 mg/kg or 60 mg/kg was comparable (Figure 3.17) and no statistically significant difference was observed (P > 0.05, Mann-Whitney U test).

Figure 3.13. Plasma total PHT concentration versus time profiles after 30 mg/kg and 60 mg/kg FosPHT and PHT administration. Values are mean ± SEM (n = 5-7).
Figure 3.14. Dose-adjusted PHT plasma Cmax values after FosPHT and PHT administration. Values are mean ± SEM (n = 5-7).

Figure 3.15. Dose-adjusted PHT plasma AUC values after FosPHT and PHT administration. Values are mean ± SEM (n = 5-7).
Figure 3.16. PHT t½ in plasma. Values are mean ± SEM (n = 5-7).

Figure 3.17. Plasma free / total PHT concentration ratios after 60 mg/kg PHT and FosPHT administration. Values are mean ± SEM (n = 3).
3.5.2 Neuropharmacokinetic comparison of phenytoin in the cerebrospinal fluid compartment after phenytoin and fosphenytoin administration

The PHT concentration versus time profiles in CSF after FosPHT and PHT administration at 30 mg/kg and 60 mg/kg are shown in Figure 3.18. Comparison of Tmax, Cmax, AUC and t½ values after FosPHT and PHT administration reveals that there were no statistically significant differences between the two drugs (P > 0.05, Mann-Whitney U test) (Figures 3.19 - 3.21). Although at 60 mg/kg, mean AUC and t½ values show some difference between FosPHT and PHT administrated groups (Figure 3.20 and 3.21), possibly due to the great inter-subject variation, the difference was not statistically significant (P > 0.05, Mann-Whitney U test).

The CSF to plasma PHT concentration ratios were also compared (Figures 3.22 and 3.23), and no statistically significant difference was observed between the two drug groups (P > 0.05, Mann-Whitney U test).

Figure 3.18. PHT concentration versus time profiles in CSF after 30 mg/kg and 60 mg/kg FosPHT and PHT administration. Values are mean ± SEM (n = 5-6).
Figure 3.19. Dose-adjusted PHT Cmax in CSF. Values are mean ± SEM (n = 5-6).

![PHT Cmax in CSF](image)

Figure 3.20. Dose-adjusted PHT AUC in CSF. Values are mean ± SEM (n = 5-6).

![PHT AUC in CSF](image)
Figure 3.21. PHT t½ in CSF. Values are mean ± SEM (n = 5-6).

![Graph showing PHT elimination half-life for 30 mg/kg and 60 mg/kg of FosPHT and PHT.]

Figure 3.22. CSF / plasma PHT concentration ratios after 30 mg/kg PHT and FosPHT administration. Values are mean ± SEM (n = 5-6).

![Graph showing CSF/Plasma PHT concentration ratios over time for PHT and FosPHT.]
Figure 3.23. CSF / plasma PHT concentration ratios after 60 mg/kg PHT and FosPHT administration. Values are mean ± SEM (n = 5-6). At time 180 minutes and 240 minutes, CSF / plasma concentration ratios were higher in 60 mg/kg PHT group than that in 60 mg/kg FosPHT group (P < 0.05, Mann-Whitney U test).

3.5.3 Neuropharmacokinetic comparison of phenytoin in brain extracellular fluid compartment after phenytoin and fosphenytoin administration

Neuropharmacokinetic characteristics of PHT in brain ECF compartment after FosPHT and PHT administration was studied at 60 mg/kg only due to the technical limitation of measuring low concentrations in microdialysate. The results revealed that, both drugs took approximately 30 minutes to reach peak concentrations, with Cmax values of approximately 10 µmol/L (Figures 3.24 and 3.25). Statistical analysis revealed that there was no statistically significant difference in Tmax, Cmax, AUC and t½ values in different brain areas (frontal cortex and hippocampus) when FosPHT and PHT administration were compared (data as shown in Tables 3.5, 3.6, 3.11 and 3.12).
Figure 3.24. PHT concentration in frontal cortex ECF versus time profiles after 60 mg/kg FosPHT and PHT administration. Values are mean ± SEM (n = 5-7). There is no difference of PHT concentration at each time point after PHT and FosPHT administration (P > 0.05, Mann-Whitney U test).

Figure 3.25. PHT concentration in hippocampal ECF versus time profiles after 60 mg/kg FosPHT and PHT administration. Values are mean ± SEM (n = 5-7). There is no difference of PHT concentration at each time point after PHT and FosPHT administration (P > 0.05, Mann-Whitney U test).
Figure 3.26. Frontal cortex ECF / plasma PHT concentration ratios after 60 mg/kg PHT and FosPHT administration. Values are mean ± SEM (n = 5-7). There is no difference at each time point after PHT and FosPHT administration (P > 0.05, Mann-Whitney U test).

Figure 3.27. Hippocampal ECF / plasma PHT concentration ratios after 60 mg/kg PHT and FosPHT administration. Values are mean ± SEM (n = 5-7). There is no difference at each time point after PHT and FosPHT administration (P > 0.05, Mann-Whitney U test).
Equilibration between brain ECF and plasma compartments was achieved at the same time (30 minutes postdose) and the ECF to plasma concentration ratio was without difference when FosPHT and PHT administration were compared (P > 0.05, Mann-Whitney U test) (Figure 3.26 and 3.27). These results indicate that the neuropharmacokinetic characteristics of FosPHT and PHT in the brain ECF compartment are indistinguishable.

3.6 Discussion

SE, particularly convulsive SE, is a medical emergency, which requires immediate medical intervention. The mortality of SE is approximately 20%, and this rises to over 50% if patients do not respond to initial therapy (DeLorenzo et al., 1996). Permanent neurological and mental damage can result from SE with the risks of morbidity being greatly increased the longer the duration of the episode. Thus a rapid and effective therapeutic response is essential in the management of SE and FosPHT has been developed as a PHT pro-drug in this regard. Indeed FosPHT has been observed to be of clinical utility in SE, and as a formulation it is clearly more amenable for clinical use compared to that of the parenteral PHT formulation (Fischer et al., 1996; Pellock, 1996). However, an important consideration relates to how the penetration of PHT, derived from FosPHT, into the brain site of action, compares to that of PHT after the administration of the parenteral formulation, particularly since a recent report has suggested that PHT whole brain tissue concentrations are significantly lower after IV FosPHT compared to PHT administration (Walton et al., 1999). Therefore, this study was designed to compare the pharmacokinetics of PHT in plasma, and the neuropharmacokinetics in CSF and brain ECF (frontal cortex and hippocampus) after IV administration of PHT and
FosPHT. This was achieved by use of a well-validated freely behaving rat model (Lolin et al., 1994; Nagaki et al., 1999; Doheny et al., 1999; Walker et al., 1996, 2000; Tong et al., 2001). This model uniquely allows concurrent and temporal pharmacokinetic-neuropharmacokinetic profiles in blood, CSF and brain ECF compartments in individual rats to be studied. Brain ECF monitoring was achieved by microdialysis which is considered to reflect events at the site of drug action. That minimal animal handling occurs during sampling, using this model, results in less induced stress and therefore more physiologically relevant kinetic data.

The pharmacokinetics of PHT in plasma after IV administration of PHT and FosPHT are indistinguishable and indeed the concentrations versus time profiles are essentially superimposable (Figure 3.13). In particular, at 5 minutes post IV administration of PHT and FosPHT, plasma PHT concentrations are identical. Furthermore, the classical well-characterised dose-dependent saturation kinetics of PHT (Eadie et al., 1976; Wilson et al., 1976; Jacobsen et al., 1986; Rundfeldt et al., 1993; Shepard et al., 1993; Lolin et al., 1994) was seen after both PHT and FosPHT administration, with comparable increases in $t_{1/2}$ values at the higher dosage.

As FosPHT is a pro-drug of PHT, it needs to be converted to PHT in order to have a pharmacological effect. Whilst the conversion half-life in man is 8-15 minutes, it is reported to be only 1 minute in the rat (Jamerson et al., 1990; Walton et al., 1999). In the present study, because the IV infusion process itself took on average 2 minutes and since by the time of first sampling (5 minutes) PHT concentrations in blood after FosPHT administration were identical to those obtained after PHT administration, it was not possible to ascertain the exact conversion half-life of FosPHT. Additionally, as no blood sample was taken at 2.5 minutes, it is not possible to confirm that at that time plasma PHT concentrations are significantly
lower after FosPHT compared to PHT administration, as reported by Walton et al (1999). Similarly it is not possible to confirm that at 2.5 minutes the free / total PHT concentration ratio was significantly greater for FosPHT compared to that of PHT (Walton et al., 1999). However, at subsequent time points (5-60 minutes) the PHT free / total PHT concentration ratio was indistinguishable after PHT and FosPHT administration and confirms that reported by Walton et al (1999). Furthermore the values of free / total PHT concentration ratio were similar to those previously reported (Lai et al., 1995; Lolin et al., 1994; Porter & Layzer, 1975; Walker et al., 1996).

As in the case of the blood compartment, the neuropharmacokinetics of PHT after PHT and FosPHT in the CSF compartment was indistinguishable. Rapid PHT CSF penetration was observed after the administration of both PHT and FosPHT with Tmax values of 9-13 minutes. Furthermore, PHT equilibration between the blood and CSF compartments occurred at approximately 15 minutes post administration of both PHT and FosPHT (Figures 3.22 and 3.23). The dose-dependent increase in CSF concentrations suggests that transport across the blood-brain barrier is not rate limiting over the concentration range observed in this study, which is in contrast to that previously reported by Lolin et al. (1994).

Although the CSF compartment has been identified as a compartment that is neuropharmacokinetically indistinguishable from that of the site of action of drugs that have an effect on the CNS, including numerous AEDs (e.g. CBZ, PB, DZP, and oxazepam), some drugs, including PHT, have been reported not to be uniformly distributed within the brain (Sechi et al., 1989; Walker et al., 1996). Consequently, microdialysis monitoring of ECF in specific brain sites has been used as a more appropriate index of drug distribution at putative sites of drug action.
In the present study frontal cortex and hippocampal ECF was monitored. After PHT and FosPHT administration PHT rapidly appeared in the brain as evidenced by the fact that PHT was detectable in the ECF of both brain regions at the time of first sampling (10 minutes). Furthermore, not only were there no differences in the neuropharmacokinetic profiles of PHT after PHT and FosPHT administration but also, in addition, there were no brain regional differences as neuropharmacokinetic parameters in the frontal cortex and hippocampus were indistinguishable. These results suggested that both drugs would be equally efficacious in the treatment of seizures and SE. Indeed this is the case since the anticonvulsant effect of FosPHT and PHT in amygdala-kindled rats is identical (Loscher et al., 1998).

The present study indicated that T_max in the ECF was longer than that for the CSF. Delayed T_max in ECF compartment suggests that PHT penetration into CSF and ECF compartments might involve different mechanisms (Sechi et al., 1989). The extracellular space of the brain constitutes the environment of the neuronal and glial cells (Woodbury, 1958). The content of ECF is primarily determined by the transport through the brain capillary endothelial cells (which form the BBB) and in part by the CSF, with which it is in intimate and rapid communication (Cornford, 1986). The composition of CSF is instead, determined by the secretary processes in choroid plexus epithelia (constitutes the blood-CSF barrier, [BCB]) (Partridge, 1986). Another possible explanation for delayed T_max in ECF compartment is that it may reflect the difference in sampling methodology in that CSF was directly sampled (and reflects point analysis) whereas ECF was not (and reflects period analysis).
Previously, using rat whole brain homogenates, Walton et al., (1999) reported that PHT concentrations were significantly greater after IV PHT administration compared to IV FosPHT administration. Those data are in contrast to the present study where PHT brain (frontal cortex and hippocampal) ECF concentrations were indistinguishable after IV PHT and FosPHT administration and emphasises the need to be cautious about whole brain drug data that may not necessarily reflect the pharmacologically relevant drug compartment.

A study of ECF PHT in human frontal cortex and hippocampus reported no regional differences within the brain (Scheyer et al., 1994a); results consistent with those observed in the present study. Furthermore another animal brain microdialysis study of PHT administrated IP showed that at a similar dose to the present study (50 mg/kg), there was no brain regional difference in relation to its neuropharmacokinetics (Walker et al., 1996). However, at 100 mg/kg, PHT concentrations in hippocampus were observed to be higher than that in frontal cortex. Whether a further dose increment, in the present study, would have revealed a difference is unknown.

In conclusion, therefore, this study has shown that, at least in the rat, there is no significant difference in the kinetics of PHT in blood, CSF and brain frontal cortex and hippocampal ECF compartments when intravenously administered PHT and FosPHT are compared. With the rapid BBB penetration and relatively slow elimination from the brain, PHT derived from FosPHT displays advantageous pharmacokinetic features that make it suitable for the treatment of SE, particularly at the early stage of SE.
4 PHARMACOKINETICS AND NEUROPHARMACOKINETICS OF TIAGABINE

4.1 Introduction

Increasing GABA concentrations in the brain synaptic cleft is one of the main strategies in suppressing brain over-excitation processes and stopping seizures. TGB, a novel GABA uptake inhibitor, acts by reducing GABA uptake into neurons and glial cells, by inhibiting the action of the GABA transporter, GAT1, and subsequently increasing brain synaptic GABA concentrations.

Although the pharmacokinetics of TGB in the blood compartment has been extensively studied in man, animal data of the pharmacokinetics of TGB in the blood compartment and indeed its neuropharmacokinetics in the CNS is very limited. Bopp's unpublished observations reveal limited information from rats and dogs (Suzdak et al., 1995), while Sills's study in rats reported on regional brain TGB concentrations (Sills et al., 2001). There are no data regarding the kinetic interrelationship of TGB between the blood and brain compartments (CSF and ECF). Information on central kinetics of TGB is only available indirectly via the observation of brain GABA changes after TGB administration. A 50% increase of ECF GABA concentration in the hippocampus was observed in a patient with partial seizures (During et al., 1992) whereas in animals systemic administration of TGB resulted in the elevation of ECF GABA in the globus pallidus with peak values 310% and 240% of basal concentrations after 21 mg/kg and 11.5 mg/kg TGB respectively. In addition ECF GABA concentrations were significantly increased in the ventral pallidum (280% increase after 11.5 mg/kg TGB and 350% increase after 21 mg/kg TGB) and substantia nigra (200% increase after 21 mg/kg TGB) (Fink-Jensen et al.,
1992). However a rat study reported that after two days of 50-200 mg/kg/day TGB administration there was no change in CSF GABA concentrations (Halonen et al 1996).

The lack of direct TGB neuropharmacokinetic data in the CNS compartment (CSF and ECF) and the inconsistent reports of TGB central effects on GABA concentrations in CSF and brain ECF compartments prompted the present study.

4.2 Methods

Males Sprague-Dawley rats weighing 250-350 g were used. Blood, CSF catheters and microdialysis probes were constructed as described in Section 2.2.1 through Section 2.2.3.

Either a blood and a CSF catheter, or a blood catheter and microdialysis probes were implanted as described in Section 2.3. Two days after the implantation of the catheters and/or probes, rats were randomly chosen to receive IP injection of TGB, at either 20 mg/kg or 40 mg/kg for rats sampled for blood and CSF or at 40 mg/kg for rats sampled for blood and microdialysate. Blood, CSF and microdialysate samples were collected at timed intervals as described in Sections 2.4.1 - 2.4.3. Total and free (non-protein bound) TGB concentrations in blood and TGB concentrations in CSF and microdialysate were analysed as described in Section 2.6.

Pharmacokinetic analysis was carried out using Microsoft Excel programme (Version 97). First order elimination was assumed and a two-compartment model was used for sera whereas a one-compartment model was used for CSF and ECF analysis as described in Section 2.10. Statistical analysis was
carried out, where appropriate, using the Mann-Whitney U test, as described in Section 2.11.

4.3 Pharmacokinetics of tiagabine in the blood compartment

TGB pharmacokinetic parameters in sera after 20 mg/kg and 40 mg/kg TGB IP administration are shown in Tables 4.1 and 4.2. After TGB administration, TGB concentrations rose dose-dependently. Cmax was reached at the time of first sampling (15 minutes postdose) for both TGB doses. TGB concentrations declined rapidly during the following 15 minutes and subsequently continued to decline exponentially (Figure 4.1). The distribution half-life was 13.4 ± 1.6 minutes and 18.2 ± 2.3 minutes for the 20 mg/kg and 40 mg/kg TGB groups respectively (P > 0.05, Mann-Whitney U test). TGB concentration values were significantly different between the two dose groups, with the 40 mg/kg group being twice that of the 20 mg/kg group. TGB Cmax values in sera were 14905 ± 1733 nmol/L for the 20 mg/kg TGB group, and 27754 ± 2000 nmol/L for the 40 mg/kg TGB group. Dose-adjusted TGB Cmax values were not statistically different between the two dose groups (P > 0.05, Mann-Whitney U test; Figure 4.2). Dose-adjusted mean AUC values in sera were approximately 20 % higher in 40 mg/kg TGB group compared to the 20 mg/kg TGB group (Figure 4.3). However, because the inter-subject variation is large, the difference is not statistically significant (P > 0.05, Mann-Whitney U test). Mean TGB t½ values were 55 ± 2.3 minutes and 50 ± 2.6 minutes for the 20 mg/kg and the 40 mg/kg TGB groups respectively and these values were not statistically different. Similarly TGB Tmax values were not significantly different when the two dose groups were compared (P > 0.05, Mann-Whitney U test).
Serum free / total TGB concentration ratios after 40 mg/kg TGB administration was calculated for one rat in the first 2 hours after TGB administration. At 15 minutes postdose, the free / total TGB concentration ratio was 0.014. Subsequently the TGB free / total concentration ratio was 0.045 ± 0.003 and was independent of serum TGB concentrations during the subsequent 90 minutes.

Table 4.1. Pharmacokinetic parameters of TGB in serum after 20 mg/kg TGB administration.

<table>
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<tr>
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<th>AUC (nmol/h L)</th>
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Table 4.2. Pharmacokinetic parameters of TGB in serum after 40 mg/kg TGB administration.

<table>
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<th>Cmax (nmol/L)</th>
<th>AUC (nmol/h L)</th>
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<td>3491</td>
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Figure 4.1. TGB concentration versus time profiles in serum after 20 mg/kg and 40 mg/kg TGB administration. Values are mean ± SEM (n = 5-7).

Figure 4.2. Dose-adjusted TGB serum Cmax values. Values are mean ± SEM (n = 5-7).
Figure 4.3. Dose-adjusted TGB serum AUC values. Values are mean ± SEM (n = 5-7).

4.4 Neuropharmacokinetics of tiagabine in the cerebrospinal fluid compartment

TGB neuropharmacokinetic parameters in CSF after 20 mg/kg and 40 mg/kg TGB administration are shown in Tables 4.3 and 4.4. The corresponding TGB concentration versus time profiles are shown in Figure 4.4. After TGB administration, TGB rapidly penetrated into the CSF compartment and was detectable in CSF at the time of first sampling (15 minutes). Mean Tmax values were 28 ± 0.7 minutes and 32 ± 0.9 minutes for the 20 mg/kg and 40 mg/kg TGB groups respectively (P > 0.05, Mann-Whitney U test). Cmax values increased in proportion to dose and were 49 ± 6.7 nmol/L after 20 mg/kg TGB and 95 ± 5.4 nmol/L after 40 mg/kg TGB administration. Dose-adjusted Cmax values were not statistically different between the two groups (P > 0.05, Mann-Whitney U test) (Figure 4.5). Similarly, dose-adjusted AUC values (Figure 4.6) were indistinguishable when the two dose groups were compared (P > 0.05, Mann-Whitney U test). However, t½ values were significantly longer after 40 mg/kg TGB administration (64 ± 2.7
minutes) compared to the values after 20 mg/kg TGB administration (40 ± 2.6 minutes) (P < 0.01, Mann-Whitney U test).

On the basis of CSF / serum TGB concentration ratio, TGB concentrations were observed to equilibrate between the blood and CSF compartments by 30 minutes postdose after 20 mg/kg TGB (Figure 4.7). However, in the TGB 40 mg/kg group, TGB concentration equilibration was achieved at 30-180 minutes postdose. From 180 minutes onwards, the CSF to serum TGB concentration ratio rose with time (Figure 4.7). The mean CSF to serum TGB AUC ratio was 0.0052 ± 0.0003 in the 20 mg/kg TGB group and 0.0056 ± 0.0006 in the 40 mg/kg TGB group and the values were not statistically different (P > 0.05, Mann-Whitney U test).

Table 4.3. TGB neuropharmacokinetic parameters in CSF after 20 mg/kg TGB administration.

<table>
<thead>
<tr>
<th>Rat No</th>
<th>Tmax (min)</th>
<th>Cmax (nmol/L)</th>
<th>AUC (nmol h/L)</th>
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</table>

Table 4.4. TGB neuropharmacokinetic parameters in CSF after 40 mg/kg TGB administration.

<table>
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<th>Rat No</th>
<th>Tmax (min)</th>
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Figure 4.4. TGB concentration versus time profiles in CSF after 20 mg/kg and 40 mg/kg TGB administration. Values are mean ± SEM (n = 5-6).

Figure 4.5. Dose-adjusted TGB Cmax values in CSF. Values are mean ± SEM (n = 5-6).
Figure 4.6. Dose-adjusted TGB AUC values in CSF. Values are mean ± SEM (n = 5-6).

Figure 4.7. CSF / serum TGB concentration ratios after TGB administration at 20 mg/kg and 40 mg/kg. Values are mean ± SEM (n = 5-6). In 20 mg/kg TGB group, TGB concentrations in serum and CSF declined to levels beyond the limit of quantification after 270 minutes.
4.5 Neuropharmacokinetics of tiagabine in brain extracellular fluid compartment

TGB neuropharmacokinetics in brain ECF compartment were studied using 40 mg/kg TGB only due to the technical limitation of TGB HPLC assay. The microdialysis probe in vitro recovery was 10 ± 0.3% (n = 20) and this value was used to correct the measured ECF TGB concentrations. The neuropharmacokinetic parameters of TGB in brain frontal cortex and hippocampal ECF after 40 mg/kg TGB administration are shown in Tables 4.5 and 4.6. The TGB concentration versus time profiles in brain frontal cortex and hippocampal ECF are shown in Figures 4.8. After drug administration, TGB penetrated the BBB rapidly and was detectable at the time of first sampling (10 minutes postdose). Mean Cmax values were 35 ± 1 nmol/L for frontal cortex and 38 ± 2 nmol/L for hippocampus. Mean Tmax values were 41 ± 5 minutes and 34 ± 3 minutes respectively. Mean AUC values were comparable in frontal cortex (154 ± 25 nmol/L) and hippocampus (134 ± 10 nmol h/L). The corresponding t½ values were 174 ± 32 and 133 ± 9 minutes respectively. Indeed, there were no statistically significant differences in relation to the neuropharmacokinetic parameters of TGB when brain frontal cortex and hippocampus were compared (P > 0.05, Mann Whitney U test).

Comparing TGB in blood with that in brain ECF, it was observed that brain TGB took twice as long to reach Cmax. Furthermore, it took three times longer than that in serum for TGB to be eliminated. ECF to serum TGB concentration ratios increased over time and therefore TGB in the blood and brain compartments did not achieve equilibration (Figure 4.9).
Table 4.5. Neuropharmacokinetic parameters of TGB in brain frontal cortex ECF after 40 mg/kg TGB administration.

<table>
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<tr>
<th>Rat No</th>
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Table 4.6. Neuropharmacokinetic parameters of TGB in brain hippocampal ECF after 40 mg/kg TGB administration.

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Figure 4.8. TGB concentration versus time profiles in brain frontal cortex and hippocampal ECF after 40 mg/kg TGB administration. Values are mean ± SEM, n = 5.

Figure 4.9. ECF / serum TGB concentration ratios in brain frontal cortex and hippocampus after 40 mg/kg TGB administration. Values are mean ± SEM, n = 5.
4.6 Discussion

The present study is the first to investigate the relationship between TGB pharmacokinetics in the blood compartment and TGB neuropharmacokinetics in the CSF and ECF compartments. The major findings of this study in rats are as follows: (1) the serum pharmacokinetics of TGB are linear in that Cmax and AUC values are proportionate to dose; (2) the TGB serum free / total concentration ratio is 0.045 and protein binding by TGB in serum is 95%; (3) t½ is 50 - 55 minutes in the blood compartment and is concentration independent; (4) the neuropharmacokinetics of TGB in the CSF compartment shared the same pattern as that in the blood compartment; (5) TGB rapidly penetrated the BBB, with the Tmax in the ECF compartment being 34 - 41 minutes; (6) TGB t½ values in the ECF compartment is 3 times longer than that in the blood and CSF compartments; (7) there was no regional difference of TGB distribution when frontal cortex and hippocampus were compared.

Data of the pharmacokinetics of TGB in animal models is sparse and are only available from the unpublished data by B. Bopp of Abbott Laboratories (Suzdak et al., 1995). TGB is rapidly absorbed after oral administration, with Tmax of 0.75-2.2 hours. The bioavailability of TGB is 25% in rats and 54% in dogs. However, because the first-pass metabolism is saturable, the bioavailability increases with repeated doses. Plasma protein binding is 92-93% in rats and 91-93% in dogs. After the administration of [14C]TGB, the levels of radioactivity in most tissues was similar to the plasma radioactivity levels; only the eliminating organs (liver, kidney) had considerably higher levels (7-10 fold). The decrease in the tissue levels of [14C]TGB followed the decrease in the plasma levels. The brain / plasma ratio remained constant at 0.24 in rats while in dogs, the ratio increased from 0.4 (30 minutes after [14C]TGB administration) to 1.0 (4 hours after [14C]TGB administration), indicating
a slow elimination from the brain or slow equilibration between plasma and brain. After IV administration, the \( t^{1/2} \) was 0.47 hours in male rats and 1.04 hours in female rats and increased to 2.4 hours in male rats and 4.5 hours in female rats when a higher dose of TGB was administered (40 mg/kg, Suzdak et al., 1995).

The findings in the present study are generally comparable to those of Bopp. In particular the serum protein binding of TGB was approximately 95% in the present study compared to 92-93% reported by Bopp. Furthermore the \( t^{1/2} \) in ECF was 3 times longer than that observed in the blood compartment which corroborated the observation by Bopp that TGB was associated with slower elimination from the brain. Lastly that the \( t^{1/2} \) value in the CSF compartment appeared to be dose-dependent (64 ± 2.7 minutes for the 40 mg/kg TGB group versus 40 ± 2.6 minutes for the 20 mg/kg TGB group, \( P < 0.05 \), Mann-Whitney U test) also corroborated the observation by Bopp.

The rate of penetration of drugs including AEDs into CSF and brain is considered to be determined essentially by three physiochemical characteristics, namely degree of ionisation, lipid solubility and degree of plasma (serum) protein binding. In relation to AEDs, lipid solubility appears to be the major determinant of rate of entry into the CSF compartment (Loscher et al., 1984). This is the case with TGB, which with its high lipid solubility, rapidly appeared in the CSF and ECF compartments. TGB was detectable in both CSF and ECF compartments at the time of first sampling (10-15 minutes postdose). The \( C_{\text{max}} \) in CSF and ECF was achieved at only 15 minutes after the serum \( C_{\text{max}} \) was achieved. There was no difference in \( T_{\text{max}} \) values between the 20 mg/kg and 40 mg/kg groups in the CSF compartment, which indicate that TGB transportation across the BCB in the choroid plexus and
BBB in the frontal cortex and hippocampus was not rate limiting at the concentration ranges achieved in the present study.

Free serum drug concentration is often used as a guide to the drug concentration in the CNS. In the present study, the CSF / serum concentration ratios and ECF / serum concentration ratios are much lower than the TGB free / total concentration ratio in serum. The disparity between these ratios has several implications: [1] that the serum free (non-protein bound) / total drug concentration ratio for TGB is not an accurate reflection of central concentrations in relation to peripheral concentrations in blood compartment; [2] that the BCB and BBB limit the entry of TGB into the CSF and ECF compartment. These data therefore emphasise the importance of studying the neuropharmacokinetics of a drug so as to establish the pattern of drug penetration and disposition at the site of drug action.

That the CSF / serum TGB concentration ratio and the ECF / serum concentration ratio rose over time, suggest that central and peripheral compartments did not achieve equilibrium. Furthermore, ECF TGB t½ values were three times longer than that for serum. These data suggested that TGB has a slower elimination rate from the brain and perhaps better brain affinity. This feature could be of important clinical implication in that the pharmacological effect of TGB at the site of drug action is far beyond what its blood pharmacokinetics would suggest. A further consideration would be the possibility for TGB accumulation in the CNS.

Three studies have reported on the effect of TGB on regional brain GABA concentrations. The study of Fink-Jensen et al. (1992) in rats reported that GABA concentration rose by 310% in globus pallidus, 350% in ventral pallidum and 200% in substantia nigra after 21 mg/kg TGB IP administration. The study of Dalby (2000) reported that GABA concentration rose in different scale in hippocampus
(645%) and thalamus (409%) after infusion of 30 mM TGB through the microdialysis probe. However, Sills et al (2001) reported that TGB was without effect on GABA concentration in different brain regions after 50 mg/kg TGB IP administration. The difference of their results might be the result of different methodology as Sills et al. (2001) used regional brain tissue homogenates while the other two studies used in vivo microdialysis. As the present study did not correlate TGB and GABA content in the ECF compartment in different brain regions, it was not possible to ascertain any brain regional differences that would have been attributed to TGB and GAT1 distribution.

In conclusion, this study has shown that TGB has favourable pharmacokinetic and neuropharmacokinetic characteristics. In particular, TGB has linear kinetics in both blood and CSF, and CNS penetration is rapid. TGB distribution in the brain, as ascertained for the hippocampus and frontal cortex, is not brain region specific. The relatively slow elimination of TGB from the brain ECF compartment maybe a potential important therapeutic advantage since its action will outlast its presence (has a short $t_{1/2}$) in blood.
5 CHARACTERISATION OF A REFRACTORY MODEL OF
STATUS EPILEPTICUS

5.1 Introduction

Having investigated the pharmacokinetics and neuropharmacokinetics of PHT, FosPHT and TGB in the blood, CSF and brain ECF, the subsequent study assessed the anticonvulsant efficacy of FosPHT, TGB and TPM in an experimental model of refractory SE as well as their neuroprotective effects. For this purpose, a perforant path stimulation induced SE model as described by Walker et al. (1999) was chosen. It is a refractory model (Holtkamp et al., 2001) and the resultant neuronal damage resembles that of human hippocampal sclerosis (Cock et al., 2002; Sloviter et al., 1996). In order to quantify the anticonvulsant efficacy and degree of neuroprotection, it was first necessary to characterise the SE model in relation to behavioural, electrographic and histological changes.

Imbalance between excitatory and inhibitory neurotransmission in the CNS has been proposed as the mechanism underlying epileptogenesis (Meldrum, 1994). During seizures, the brain exhibits highly synchronised neuronal excitation. However, the way in which seizures and SE are initiated and maintained is still a mystery. Numerous studies in humans and animals have been carried out using microdialysis techniques to investigate the pattern of change of neurotransmitters and other amino acids during seizures and SE. Glutamate and GABA, as the main excitatory and inhibitory neurotransmitters respectively, are the most commonly studied amino acids. Human studies reported relatively consistent findings that during seizures, extracellular concentrations of glutamate, GABA, aspartate and taurine significantly increase (Carlson et al., 1992; During et al., 1993; Wilson et al.,
1996). In particular, glutamate concentrations rise prior to seizure onset (During et al., 1993). Studies in animal models of seizures and SE have produced inconsistent results. Increased glutamate concentration during seizures was observed in numerous studies (Lallement et al., 1991; Khan et al., 1999; Wilson et al., 1996; Zhang et al., 1990), whereas decreased glutamate concentration and increased GABA concentration during SE was observed by Walton et al., (1990c) and Cavalheiro et al., (1994). Therefore, the second aim of this study was to characterise the pattern of GABA, glutamate, taurine and other amino acid neurotransmitter change in the hippocampal ECF during the process of SE induction and maintenance. This was achieved by intracerebral microdialysis.

5.2 Methods

Male Sprague-Dawley rats (280-330g) were implanted with an electrode-microdialysis combined probe (constructed as described in section 2.2.5) according to the procedures described in section 2.3.6. Post-surgery rats were housed individually in Perspex cages until recovery. Five rats with implanted electrodes were selected randomly as the control group without perforant path stimulation. A further 5 rats had perforant path stimulation as described in section 2.8.2 at two days after the probe implantation. The rats were kept alone and freely moving around in individual cages (40cm x 20 cm x 40cm) during the process of perforant path stimulation and subsequent recording. A cable, going through a hole at the centre of the cage lid, with one end attached to the rat's head and the other end attached to a freely moving swivel, with its weight counterbalanced, delivered the stimulation currents and electrographic activity recording to and from the rat. During the post
perforant path stimulation observation period, the EEG was sampled for one minute in every ten-minute time window and analysed offline.

Concurrent microdialysis of hippocampal ECF was carried out 0.5 - 1 hour prior to the beginning of perforant path stimulation and continued until 3 hours after its termination as described in section 2.4.4. Dialysate samples were stored at -70 °C until analysis for amino acid content, as described in section 2.7.

Ten minutes after the end of perforant path stimulation, rats in SSSE were injected IP with 1 ml saline. At 3 hours after the end of perforant path stimulation rats that were still seizing were injected with 50 mg/kg propofol to terminate seizures. This was achieved within 20 minutes. Subsequently, all rats were administered 5 ml saline subcutaneously so as to assist rehydration and were then kept in their individual cages. Fourteen to seventeen days later both saline treated SSSE rats and unstimulated (control) rats were prepared for histological study as described in section 2.9. As no difference in the size of hippocampal structures between control and SSSE rats had been found in previous studies conducted using the same perforant path stimulation induced SE model (personal communication with the researcher - Michelle Noble), this study assumed that changes in neuronal density in SSSE rats would be attributed to the effect of SE. A Mann-Whitney U test was carried out as appropriate and a P value < 0.05 was regarded as statistically significant.
5.3 Results

5.3.1 Electrographic and behavioural changes

Figure 5.1 shows a single dentate granule cell field potential recorded following a single pulse perforant path stimulation. During repeated stimulation hyperexcitability occurred with multiple population spikes (Figure 5.2). After approximately 30-50 minutes spontaneous large amplitude discharges started to develop and were sustained throughout the rest of perforant path stimulation period.

Behavioural observation showed that rats responded to perforant path stimulation instantly and started to exhibit typical limbic seizures with stop and staring, facial movement, forelimb clonus, rearing, even rearing and falling. Seizure severity ranged from stage 1-5 (most common at 2-4) (Racine classification, Racine 1972). Seizures usually continued for 5-10 minutes, followed by a 'silent' period when rats had wet dog shakes or wandered around in the cage. Seizures and 'silent' periods alternated in a cyclic pattern. As perforant path stimulation was continued, seizure periods became longer while 'silent' periods became shorter. As early as 45 minutes after commencing perforant path stimulation, rats started to have continuous stage 3-5 seizures with complete disappearance of 'silent' periods.

Figure 5.1. Dentate granule cell field potential. Field potential recorded from dentate granule cell layer following single pulse stimulation of the perforant path.
Figure 5.2. Multiple population spikes during stimulation. Arrows denote the point of stimulation, which is then followed by multiple population spikes.

After the end of the perforant path stimulation, all 5 rats continued to exhibit behavioural and EEG epileptic activity (spikes) that sustained for more than three hours. Rats with EEG spikes > 0.1 Hz were grouped as SSSE rats. Rats in SSSE lost auditory startle reflex and had continuous stage 1 - 4 seizures (Racine, 1972). EEG spikes sustained at an average of 2.1 ± 0.3 Hz (1.1-3.2 Hz), while EEG spike amplitude was at an average of 1.2 ± 0.43 mV (0.7 - 2.5 mV). In SSSE rats the spike amplitude and frequency were comparable at early and late stage of observation period with no significant difference (P > 0.05, Mann-Whitney U test) (Figure 5.3). This suggested that in the perforant path stimulation induced SE model, SE severity did not decline significantly over time during the study period (0 - 180 minutes after termination of perforant path stimulation in this study). An EEG of an unstimulated control and a SSSE rat are shown in Figure 5.4.
Figure 5.3. Percentage change of spike frequency and amplitude from baseline value (value at immediately after perforant path stimulation was terminated, value = 100%) in saline treated SSSE rats at different time windows after the end of perforant path stimulation (values are mean ± SEM, n = 5).

The values of spike frequency/amplitude at different time points were pooled together in each hour. No statistically significant difference was found between values during the 1st hour, 2nd hour and 3rd hour. (P > 0.05, Mann-Whitney U test).

5.3.2 Histology

The histology of an unstimulated control rat and a saline treated SSSE rat is shown in Figure 5.5. First, the intra-observer reliability was assessed by counting the same areas on different days and comparing the neuronal density. No significant difference between the values at the same areas in different counts was found (P > 0.05, Mann-Whitney U test). This suggested that the counting method employed in the study was reproducible.
Figure 5.4. EEG of a control rat (unstimulated) and a saline treated SSSE rat at different stages of seizure development.
Visual inspection (magnification x 2.5) revealed significantly less neurons in the hilus region in the saline-treated SSSE rats than that for unstimulated control rats, whereas CA1 and CA3 cell body layers from both groups were comparable. However, under a high-power microscope (x 100 for neuron counting), saline-treated SSSE rats exhibited approximately 40% - 50% neuronal cell loss in CA1, CA3 and hilus regions (Table 5.1). There was no difference in neuronal density between the left and right side of the hippocampus (P > 0.05, Mann-Whitney U test; Figure 5.6). The neuronal density was compared between saline-treated SSSE rats and unstimulated control rats. The former displayed significantly less neurons than the latter in all three hippocampal regions investigated (P < 0.01, Mann-Whitney U test) (Figure 5.7).

Table 5.1. Neuronal density (in 20 μm x 20 μm x 5 μm boxes) in hippocampal CA1, CA3 and hilus in control rats and saline treated SSSE rats. Values are mean ± SEM, n = 5.

<table>
<thead>
<tr>
<th></th>
<th>CA1</th>
<th>CA3</th>
<th>Hilus</th>
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<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>Control</td>
<td>15.7</td>
<td>15.1</td>
<td>11.9</td>
</tr>
<tr>
<td></td>
<td>± 0.58</td>
<td>± 0.48</td>
<td>± 0.43</td>
</tr>
<tr>
<td>Saline treated</td>
<td>9.0</td>
<td>8.9</td>
<td>6.9</td>
</tr>
<tr>
<td>SSSE rats</td>
<td>± 0.76</td>
<td>± 0.43</td>
<td>± 0.48</td>
</tr>
</tbody>
</table>
Figure 5.5. Histology of hippocampus (x 2.5) and close up of CA1 (x 40) from an unstimulated control rat and a saline treated SSSE rat.
Figure 5.6. Neuronal density (in 20 μm x 20 μm x 5 μm boxes) at the left and right side of the hippocampus in the saline treated SSSE group. Values are mean ± SEM, n = 5. There was no significant difference in neuronal density between the left and right side of CA1, CA3 and hilus (P > 0.05, Mann-Whitney U test).

Figure 5.7. Neuronal density (in 20 μm x 20 μm x 5 μm boxes) in three regions of the hippocampus in the unstimulated control group and the saline treated SSSE group. Values are mean ± SEM, n = 5. There were significantly less neurons in all three hippocampal regions of the saline-treated SSSE group compared to that in the unstimulated control group (P < 0.01, Mann-Whitney U test)
5.3.3 Amino acids in brain extracellular fluid

Due to technique difficulties microdialysis of hippocampal ECF amino acids was only successful in 3 out of 5 SSSE rats studied. With the onset of perforant path stimulation and the subsequent limbic seizures, hippocampal ECF glutamate concentrations rose slightly and briefly at 30 minutes then declined steadily by approximately 50% until the end of perforant path stimulation. The decline was significant from 75 minutes onwards when compared with those before perforant path stimulation (P < 0.05, Mann-Whitney U test). ECF GABA concentrations rose steadily and compared to baseline values reached statistical significance from 30 minutes onwards. They plateaued at 75 minutes and remained at higher concentrations (up to 220% of baseline concentrations) throughout the perforant path stimulation period (values during stimulation versus baseline values, P < 0.01, Mann-Whitney U test). ECF taurine concentrations declined gradually by up to 50% of baseline concentrations (P < 0.01, Mann-Whitney U test, Figure 5.8).

After the end of perforant path stimulation, glutamate concentrations returned to baseline values and were sustained for about 60 minutes but subsequently showed slight fluctuation (values in 180-minute post perforant path stimulation period versus baseline values, P > 0.05, Mann-Whitney U test). During the same period, GABA concentrations gradually declined and by 180 minutes post perforant path stimulation they were comparable to baseline values. However, in the first 75 minutes post perforant path stimulation, they were still higher than baseline values (P < 0.01, Mann-Whitney U test). Taurine concentrations remained lower than baseline values throughout the study period (P < 0.01, Mann-Whitney U test) (Figure 5.8).

With regard to other amino acids (glycine, alanine and glutamine), no difference was observed between the pre-perforant path stimulation period, perforant
Figure 5.8. Percentage change of glutamate, GABA and taurine concentrations in relation to baseline (time = 0 minute, values = 100%) in the hippocampal ECF during (time = 0-120 minutes) and after perforant path stimulation (time = 121 – 300 minutes). Values are mean ± SEM, n = 3. Reduction of glutamate concentrations is significant between 75 and 120 minutes (P < 0.05, Mann-Whitney U test); Reduction of taurine concentrations is significant between 30 and 300 minutes (P < 0.01, Mann-Whitney U test); Increase of GABA concentrations is significant between 30 and 195 minutes (P < 0.01, Mann-Whitney U test).
Figure 5.9. Percentage change of glycine, alanine and glutamine concentrations in relation to baseline (time = 0 minute, values = 100%) in the hippocampal ECF during (time = 0-120 minutes) and after perforant path stimulation (time = 121 – 300 minutes). Values are mean ± SEM, n = 3. Glycine, alanine and glutamine concentrations did not differ significantly from the baseline values (P > 0.05, Mann-Whitney U test).
path stimulation period and during the 180 minutes post perforant path stimulation (SSSE) period (P > 0.05, Mann-Whitney U test) (Figure 5.9).

5.4 Discussion

This study showed that (1) 120-minute perforant path stimulation induced SE model is stable in that the SE severity did not decline over the first 3 hours post perforant path stimulation; (2) SSSE caused significant neuronal loss in hippocampal CA1, CA3 and hilus; (3) hippocampal neuronal loss were comparable in the stimulated and the contralateral side; (4) during perforant path stimulation, hippocampal ECF GABA concentrations rose significantly while glutamate and taurine concentrations declined; (5) after termination of perforant path stimulation, hippocampal ECF GABA and glutamate concentrations gradually returned to baseline values while taurine concentrations remained depressed; (6) hippocampal ECF glycine, alanine and glutamine concentrations during perforant path stimulation and in SE were comparable to that of baseline values.

The specific pattern of cell death occurring in the hippocampus following SE has been well documented in humans (DeGiorgio et al., 1992) and in various animal models (Wasterlain et al., 1993). In the present study the histological findings were similar to those observed in previous animal studies (Sloviter, 1987, 1996; Pitkanen et al., 1996; Walker et al., 1999; Cock et al., 2002) and in human SE, with neuronal loss in a characteristic distribution, particularly affecting hippocampal CA1, CA3 and hilus.

This study indicated that the chosen refractory SE model was suitable to study the anticonvulsant efficacy of AEDs as the severe electrographic activity post
perforant path stimulation was persistent. Furthermore the similarity of histological outcome between the chosen SE model in this study and human SE rendered the study clinically useful.

Imbalance between brain excitatory and inhibitory neurotransmitters has been proposed as the mechanism underlying epileptogenesis (Meldrum, 1994). Using microdialysis technique, changes in extracellular neurotransmitter amino acid concentrations were used as indirect evidence of neurochemical modifications during seizures in several animal models. However, numerous attempts to clarify the role of extracellular amino acids in seizure activities provided various results (Smolders et al., 1997; Cavalheiro et al., 1994; Rowley et al., 1995). This study is the first to provide in vivo data concerning changes in hippocampal extracellular GABA, glutamate, taurine and other amino acids in freely moving rats in the process of SE induction and maintenance in perforant path stimulation induced SE model.

The findings in this study are in agreement with the results from Cavalheiro's and Walton's studies in the SE model induced by lithium and pilocarpine IP injection (Cavalheiro et al., 1994; Walton et al., 1990[c]). In Cavalheiro's study pilocarpine injection was associated with three-phase changes, namely acute (SE) phase, silent (seizure-free) phase and chronic (spontaneous recurrent seizures) phase. In the acute phase, when rats exhibited repetitive limbic seizures and SE, there was a drop in aspartate and glutamate concentrations and a rise in GABA concentrations. Glutamate concentrations decreased during SE and remained low at 24 hour after pilocarpine administration. GABA concentrations were high during the SE period and decreased below control group values 24 hour after pilocarpine administration. In Walton's study,
glutamate concentrations were high prior to status but decreased to concentrations significantly lower than control from early to late status; GABA concentrations increased gradually and from the mid status period the GABA increases reached statistical significance (Walton et al., 1990[c]).

The decreased glutamate and increased GABA concentrations during seizures and SE observed in the present study and in Walton's and Cavalheiro's study in different SE models (Walton et al., 1990[c]; Cavalheiro et al., 1994) are interesting, as this pattern of change of excitatory and inhibitory neurotransmitters seems to disagree with the concept that glutamate concentrations increased while GABA concentrations decreased during seizures (During et al., 1993). GABA and glutamate concentrations in brain synaptic clefts are determined by the amount of synaptic release and the re-uptake into neurons and glial cells. Synaptic release could be increased or decreased whereas uptake of glutamate and GABA in the synaptic clefts into neurons and glial cells could be up- or down-regulated by different mechanisms. In the present study, GABA concentrations started to rise after the initiation of perforant path stimulation and lasted into the early phase in the post perforant path stimulation period (75 minutes post perforant path stimulation). Increase of the ECF GABA concentrations might be a response to perforant path stimulation, acting as a compensatory mechanism to suppress the firing of glutamatergic neurons so as to maintain the balance between excitation and inhibition. However, prolonged elevation of GABA concentrations in the brain ECF would result in various events: (1) down-regulation / desensitisation of GABA_A receptors (Krnjevic, 1990; Jackson et al., 1994; Overstreet et al., 2000) and fading of GABA inhibitory effect (Krnjevic, 1990); (2) feedback inhibition of GABA release due
to the activation of presynaptic GABA<sub>B</sub> receptors (Jackson et al., 1994; Davies et al., 1990, 1993; Mott et al., 1993; Brucato et al., 1995); (3) inhibition of interneurons through tonic inhibition of GABA<sub>A</sub> receptors (Semyanov et al., 2003; Overstreet et al., 2001); (4) activation of GABA<sub>A</sub> receptor and the production of both hyperpolarising and depolarising responses (Alger et al., 1982; Grover et al., 1993; Jackson et al., 1994); (5) tonic inhibition of the synaptic glutamate release via the activation of presynaptic GABA<sub>B</sub> receptors (Rowley et al., 1995; Bonnano et al., 1992; Gallo et al., 1981; Overstreet et al., 2001), which would possibly explain the observation in this thesis that brain ECF glutamate concentrations decreased while GABA concentrations increased >2 fold during perforant path stimulation, and when GABA concentrations gradually declined to baseline values during the post perforant path stimulation period glutamate concentrations also rose to baseline values. The inverse relationship between glutamate and GABA concentrations in the brain ECF could also be explained by the correlated biochemical pathway of synthesis and metabolism between glutamate and GABA (Hassel et al., 1998).

Taurine is generally regarded as an inhibitory neurotransmitter (Durelli et al., 1983). The main action of taurine is osmoregulation (Huxtable, 1992). It probably exerts a depressant action on membrane excitability and also regulates brain glutamate concentrations (Durelli et al., 1983). In the present study, taurine concentrations decreased after the start of electrical stimulation and were reduced throughout the period with seizures and SE. These observations are in agreement with another rat study in which taurine concentrations were observed to decrease during pentylenetetrazol elicited seizures (Li et al., 2000). However, taurine concentrations have been reported to
increase during soman- and kainic acid-induced seizures (Vezzani et al., 1985; Wade et al., 1987). Thus the role of taurine in epileptogenesis and seizure maintenance requires further study.

In summary, this study explored the excitatory and inhibitory neurotransmitters in the brain hippocampal ECF during and after perforant path stimulation, and observed neurotransmitter imbalance between glutamate and GABA concentrations in the process of seizure and SSSE, which might be explained by GABA hyperfunction in attempts to suppress the over-excitation process induced by electrical stimulation. However, due to time constrains and methodological difficulties the number of animals studied is small and therefore more experiments will be necessary to confirm the above findings in this exploratory study.
6  EFFICACY OF FOSPHENYTOIN, TIAGABINE AND

TOPIRAMATE IN THE TREATMENT OF EXPERIMENTAL

REFRACTORY STATUS EPILEPTICUS

6.1 Introduction

Timely and effective treatment at the early stage of SE is vital so as to
prevent neuronal damage that may result in delayed development of chronic intractable
epilepsy. Treatment with conventional first line AEDs (DZP, PHT, PB) terminates SE in
just 50-60% of cases (Treiman et al., 1998). In the case of LZP termination of SE was
achieved in 64.5% of patients. For patients in SE refractory to these AEDs, alternative
treatment is required.

TPM is indicated for the treatment of simple partial, complex partial and
secondary generalised seizures. Its efficacy in refractory SE has rarely been studied in
humans and animals. One anecdotal report described that 800 mg TPM stopped seizures
completely in a patient with complex partial SE that was resistant to PHT, DZP, CBZ,
clobazam, paraldehyde and thiopentone (Reuber et al., 2002). Although this was a single
case it provided some evidence that TPM might be effective in the treatment of
refractory SE.

TGB is indicated in the treatment of complex partial seizures with secondary
generalisation (Richens et al., 1992; Rowan et al., 1993; Kmiec et al., 2000). TGB
displayed protective effects from SE when it was administered before SE induction
(Halonen et al., 1996) in a rat model induced by 30 minutes perforant path stimulation. It
was also effective in a model of generalised convulsive SE induced by homocysteine
thiolactone injection (Walton et al., 1990b). There are no reports on the use of TGB in the treatment of SE in man.

Consequent to the troublesome side effects of PHT, there is now a tendency to replace PHT with FosPHT. However, to date there has been no clinical data comparing the efficacy of these two drugs in the treatment of refractory SE. PHT has been studied in a perforant path stimulation model of refractory SE with disappointing results (Holtkamp et al., 2001). Whereas in non-refractory SE model FosPHT has been reported to be less effective than PHT (Walton et al., 1990[a]).

In the present study the potency of FosPHT, TGB and TPM in terminating refractory SE was determined in perforant path stimulation induced model of SE.

6.2 Methods

Male Sprague-Dawley rats (weighing 280-350g) were implanted with stimulating and recording electrodes, and SSSE induced at 7-10 days after electrode implantation as described in Sections 2.3.5 and 2.8.2. Subsequently TGB, FosPHT and TPM were evaluated for their efficacy in the treatment of SE and also their neuroprotective effects. All drugs and vehicles (20% dimethyl sulfoxide [DMSO, Sigma-Aldrich, UK] in saline) were administered IP at 10 min after the end of perforant path stimulation.

Rats (with confirmed SSSE) were randomised to receive injections of the following drugs: 100 mg/kg FosPHT, 40 mg/kg TGB, 20 mg/kg TGB, 80 mg/kg TPM, 160 mg/kg TPM or vehicle. The injection volume was 1 ml/kg. All the drugs were
constituted in saline and 20% DMSO was added to each drug solution to enhance solubility.

After drug administration, the animals were observed continuously for 180 minutes and their EEG, seizures and behaviour were recorded. The observer was unaware of the treatment (drug or vehicle) administered. SSSE, after drug administration, was ascertained from both behavioural (seizure activities according to Racine classification) and EEG recording. EEG was simultaneously recorded via a computer running Labview software (National Instruments, UK). Spike frequency and amplitude were sampled for one minute in every ten-minute time window and analysed offline.

For animals that continued seizing or displayed epileptic activity on the EEG at 3 hours after drug administration, a bolus of propofol at 50 mg/kg was injected IP so as to terminate the SSSE. Subsequently all rats were administered 5 ml of saline subcutaneously so as to assist rehydration. Rats were then kept in their individual cages for two weeks and subsequently prepared for histology procedures.

6.3 Efficacy of vehicle treatment in the status epilepticus

Vehicle was administered to 5 SSSE rats at 10 minutes after cessation of perforant path stimulation. The EEG of one rat is shown in Figure 6.1. During the 180 minutes after vehicle administration, behavioural and EEG epileptic activity continued and remained unchanged in all the rats. Comparing vehicle treated SSSE rats to saline treated SSSE rats at corresponding time points revealed that there was no difference in spike frequency and amplitude (P > 0.05, Mann-Whitney U test) (Figure 6.2 and 6.3).
Figure 6.1. The EEG of a rat in vehicle treated group. Vehicle was administered IP at 10 minutes after the end of perforant path stimulation. ([A] Before perforant path stimulation; [B] 10 minutes after; [C] 30 minutes after; [D] 60 minutes after; [E] 120 minutes after; [F] 180 minutes after). Regular spikes (defined as upward deflections of the EEG lasting less than 70 milliseconds) are seen throughout the 180-minute period after the end of perforant path stimulation.
Figure 6.2. Percentage change of spike amplitude in relation to baseline value in vehicle and saline treated animals during the 3-hour observation period. Values are mean ± SEM, n = 5. Baseline value refers to the value at the time (0 minutes) of saline/vehicle administration.

Figure 6.3. Percentage change of spike frequency in relation to baseline value in vehicle and saline treated animals during the 3-hour observation period. Values are mean ± SEM, n = 5. Baseline value refers to the value at the time (0 minutes) of saline/vehicle administration.
This suggested that vehicle treatment did not reduce the severity of SE.

6.4 Efficacy of fosphenytoin in the status epilepticus

FosPHT (100 mg/kg) was administered to 5 rats at 10 minutes after cessation of perforant path stimulation. The EEG of a rat is shown in Figure 6.4. During the period after FosPHT administration, behavioural and EEG epileptic activity continued in all five rats, although the seizures were less severe compared to that before FosPHT. Seizure severity (in Racine classification) dropped from stage 3-4 to stage 1-2 in 3 out of 5 rats within 30 minutes after FosPHT administration and remained at this level throughout the rest of the observation period, whereas it remained unchanged in the other 2 rats (Figure 6.5). Spike amplitude decreased in all rats to 40 % - 60 % with a mean of 51 ± 2 % of their baseline amplitude (Figure 6.6). Spike frequency also decreased after FosPHT treatment, ranging from 36 % - 65 % with a mean of 50 ± 3 % of baseline frequencies (Figure 6.7). The spike amplitude and frequency were lower in the FosPHT treated group than those in the vehicle treated group (P < 0.05, Mann-Whitney U test).

During the 180 minutes after FosPHT administration, rats continued to exhibit limbic SE but all rats retained auditory startle reflex. There was no respiratory suppression or other apparent behavioural abnormality.
Figure 6.4. The EEG of a rat in 100 mg/kg IP FosPHT treated group. ([A] Before perforant path stimulation; [B] 10 minutes after; [C] 30 minutes after; [D] 60 minutes after; [E] 120 minutes after; [F] 180 minutes after).
Figure 6.5. Change in seizure severity after 100 mg/kg FosPHT administration. Values are mean ± SEM, n = 5.

Figure 6.6. Percentage change in spike amplitude in relation to baseline value in rats after 100 mg/kg FosPHT administration. Values are mean ± SEM, n = 5. Baseline value refers to the value at the time (0 minutes) of drug administration.
Figure 6.7. Percentage change in spike frequency in relation to baseline value in rats after 100 mg/kg FosPHT administration. Values are mean ± SEM, n = 5. Baseline value refers to the value at the time (0 minutes) of drug administration.

6.5 Efficacy of tiagabine in the status epilepticus

6.5.1 Tiagabine administration at 40 mg/kg

Six rats received 40 mg/kg TGB administration at 10 minutes after the end of perforant path stimulation. One rat died soon after drug administration (probably as a result of strangulation during drug administration). In the remaining five rats, both the amplitude and frequency of the EEG spikes displayed a stepwise decrease from 10 minutes after TGB administration and were aborted at 20-40 minutes after TGB administration (Figure 6.10 and 6.11). Behavioural seizures displayed a similar pattern of decrease in the severity in Racine scale and finally stopped at the same time as spikes were aborted (Figure 6.8). A typical EEG of a rat at different stages after perforant path stimulation is shown in Figure 6.9. The EEG of all five rats became spike-free and
displayed suppressed electrical activity, which is a common phenomenon after the cessation of SE. From 120 minutes after TGB administration, there were continuous slow activities at approximately 1 Hz (Figure 6.7 [E] and 6.7 [F]). Nevertheless the EEG remained spike-free.

All rats were in the state of mild sedation (grade 1-2) according to the sedation classification by Lee et al. (1998). However they retained auditory startle reflex, tail pinch reflex and corneal reflex. There was no respiratory suppression or any other apparent behavioural abnormality. During the period when EEG displayed slow activity, there were no accompanying behavioural seizures.

*Figure 6.8. Change in seizure severity after TGB administration (time = 0 minute) at 20 mg/kg and 40 mg/kg. Values are mean ± SEM, n = 5.*
Figure 6.9. The EEG of a rat in 40 mg/kg IP TGB treated group. ([A] Before perforant path stimulation; [B] 10 minutes after; [C] 30 minutes after; [D] 60 minutes after; [E] 120 minutes after - regular slow activity (less than 4 Hz); [F] 180 minutes after – slow activity continued).
Figure 6.10. Percentage change in spike amplitude in relation to baseline value in rats after 40 mg/kg TGB administration. Values are mean ± SEM, n = 5. Baseline value refers to the value at the time (0 minutes) of drug administration.

Figure 6.11. Percentage change in spike frequency in relation to baseline value in rats after 40 mg/kg TGB administration. Values are mean ± SEM, n = 5; Baseline value refers to the value at the time (0 minutes) of drug administration.
6.5.2 Tiagabine administration at 20 mg/kg

Because 40 mg/kg TGB was effective in terminating SSSE, the TGB dose was halved so as to test whether TGB was also effective at a lower dose. Thus, 5 rats received 20 mg/kg TGB administration at 10 minutes after the end of perforant path stimulation. The EEG of a rat is shown in Figure 6.12. Four of the 5 rats showed a stepwise decrease in the frequency and amplitude of the spikes with a corresponding decrease in the seizure severity as determined by the Racine scale. These rats finally stopped seizing and their EEG became spike-free, at 45 minutes post drug administration in two rats and at 70 minutes in the other two. However, this effect lasted only 10 to 20 minutes after which, EEG spikes resumed and rats displayed limbic seizures again at a reduced scale (Figure 6.8). In all 4 rats, spike recurrence was associated with smaller amplitude compared to that observed prior to drug administration (Figure 6.13). The reduction was of the order of 50% and lasted approximately 1 hour. This pattern was similarly seen with spike frequency (Figure 6.14). During this period, the spike amplitude and frequency were significantly lower in the TGB treated group compared to the vehicle treated group (P < 0.05, Mann-Whitney U test). Subsequently spike amplitude gradually returned to that observed at the time before drug administration while spike frequency remained lower than those observed at this time.

After 20 mg/kg TGB administration, mild sedation (grade 1-2) was observed and it typically lasted for 10-20 minutes. There was no respiratory suppression and behavioural abnormality was not apparent.
Figure 6.12. The EEG of a rat in 20 mg/kg TGB treated group. ([A] Before perforant path stimulation; [B] 10 minutes after; [C] 30 minutes after; [D] 60 minutes after; [E] 120 minutes after - some slow activity; [F] 180 minutes after - spikes resumed).
Figure 6.13. Percentage change in spike amplitude in relation to baseline value in rats after 20 mg/kg TGB administration. Values are mean ± SEM, n = 5. Baseline value refers to the value at the time (0 minutes) of drug administration.

Figure 6.14. Percentage change in spike frequency in relation to baseline value in rats after 20 mg/kg TGB administration. Values are mean ± SEM, n = 5; Baseline value refers to the value at the time (0 minutes) of drug administration.
6.6 Efficacy of topiramate in the status epilepticus

Six rats received 80 mg/kg TPM administration at 10 minutes after the end of perforant path stimulation. The EEG of a rat is shown in Figures 6.15. During 180 minutes after drug administration, seizures and EEG spikes continued. The seizure severity remained largely unchanged (Figure 6.16). The amplitude of EEG spikes was reduced slightly with a maximal reduction of 30% from baseline level (Figure 6.17). Spike frequency was reduced by approximately 40% compared to that seen before TPM administration (Figure 6.18). The spike amplitude and frequency after TPM administration were comparable to those in the vehicle treated group (P > 0.05, Mann-Whitney U test).

Because the effect of 80 mg/kg TPM was minimal, a dose of 160 mg/kg TPM was also investigated. Thus, 5 rats with SSSE were administered 160 mg/kg TPM. None of the rats stopped seizing and its effect on seizure severity, EEG spike amplitude and frequency was minimal (compared to those in vehicle treated rats and rats with 80 mg/kg TPM, P > 0.05, Mann-Whitney U test).

Apart from the limbic SE, rats did not display respiratory suppression or any other behavioural abnormality after TPM administration at either 80 mg/kg or 160 mg/kg.
Figure 6.15. The EEG of a rat in 80 mg/kg TPM treated group. ([A] Before perforant path stimulation; [B] 10 minutes after; [C] 30 minutes after; [D] 60 minutes after; [E] 120 minutes after; [F] 180 minutes after).
Figure 6.16. Change in seizure severity after 80 mg/kg TPM administration. Values are mean ± SEM, n = 5.

Figure 6.17. Percentage change in spike amplitude in relation to baseline value in rats after 80 mg/kg TPM administration. Values are mean ± SEM, n = 6. Baseline value refers to the value at the time (0 minute) of drug administration.
6.7 Discussion

Refractory SE is a medical difficulty demanding effective treatments and a substantial amount of research has aimed to identify these in different animal models. Using the same perforant path stimulation model of refractory SE as that used in the present study, AEDs administered immediately after the end of electrical stimulation have been associated with different efficacies. PHT 50 mg/kg failed to terminate SSSE, while DZP 5 mg/kg was effective in 40% of animals. The anaesthetic agent propofol at 50 mg/kg and pentobarbital at 60 mg/kg administered IP were effective in terminating SSSE in all animals but was associated with mild sedation (Holtkamp et al., 2001). In the present study a variety of new generation AEDs were investigated, some for the first time, and the results similarly showed different efficacy for the different drugs: (1) TGB terminated behavioural and EEG epileptic activity rapidly and completely in a dose-
dependent manner; (2) high dose FosPHT (100 mg/kg) did not stop behavioural and EEG epileptic activity but reduced their severity by approximately 50%; (3) high dose TPM (80 mg/kg and 160 mg/kg) failed to stop seizure activity or reduce the severity of behavioural and EEG epileptic activity.

SE can be induced by perforant path stimulation using different stimulation protocols: 30 minutes at 2 Hz (Mazarati et al., 1998), 60 minutes at 20 Hz (Halonen et al., 1996; Pitkanen et al., 1996), or 120 minutes at 20 Hz (Walker et al., 1999; Holtkamp et al., 2001). The longer the stimulation lasts, the more severe the SE (Mazarati et al., 1998). The potency of PHT in the SE model decreased with increasing stimulation duration from 30 minutes to 60 minutes (Mazarati et al., 1998). After 120-minute stimulation, PHT lost its potency in terminating SSSE (Holtkamp et al., 2001). These data suggest that the SSSE model induced by 120-minute perforant path stimulation (Walker et al., 1999; Holtkamp et al., 2001), and used in the present study, is a refractory model of SE.

Different SE models require different doses of AEDs for them to be effective and data in this regard are very incomplete. The ED50 of TGB in a cobalt-lesioned rat SE model induced by homocysteine thiolactone IP injection was 8.3 mg/kg (Walton et al., 1994). PHT and FosPHT were tested at 120 mg/kg in the same model where PHT achieved much better seizure control than FosPHT (Walton et al., 1990a). TPM (80 mg/kg) was used in lithium-pilocarpine induced SE model in neonate rats (Cha et al., 2002), while 20mg/kg, 40 mg/kg and 80 mg/kg TPM was used in an electrically stimulated SE model (Nieberauer et al., 1999). The AED doses used in the present study were chosen to be high both in relation to their target therapeutic concentration range.
and also in relation to other studies so that their maximal anticonvulsant potency within maximally tolerated dosage could be identified. When TGB exhibited significant efficacy in terminating SE, the dose was halved to 20 mg/kg, which displayed limited anticonvulsant potency.

Adverse side effects were closely monitored in this study. In TGB 40 mg/kg group, mild sedation was observed after behavioural and EEG epileptic activity was terminated. This could be the combined effect of TGB itself and the post-ictal suppression that usually presents in the clinical situation. No other side effects were observed in rats administered TGB 40 mg/kg, which suggested that the dose was well tolerated. TPM and FosPHT administration were not associated with any obvious side effects.

Data of the potency of FosPHT in experimental models of SE is very limited and has only been reported by Walton et al (1990). In Walton's study, FosPHT was effective in stopping generalised convulsive SE induced by homocysteine thiolactone. This is in contrast to the finding in the present study where FosPHT was without effect in aborting limbic SE. The difference may be the result of different models of SE that were used. The model used in the present study is self-sustaining and refractory, and consequently AEDs need to be particularly effective to suppress seizure activities. Indeed this pattern is similar to that seen in studies of PHT. Thus while PHT is effective in arresting SE in the homocysteine thiolactone rat model (Walton et al., 1990), the transauricular electrical stimulation mice model (Honack et al., 1992) and the perforant path stimulation model (with 30-60 minutes stimulation) (Mazarati et al., 1998), it is not very effective in refractory models of SE induced by perforant path stimulation (with
120 minutes stimulation) (Holtkamp et al., 2001), lithium-pilocarpine or kainic acid (Mecarelli et al., 1997). It is noteworthy that this pattern of AED efficacy is consistent with clinical findings whereby conventional first line AEDs terminate SE in only 50-60% of cases (Treiman et al., 1998).

TPM has been studied in various seizure models and was effective in MES test in mice and rats (Kimishima et al., 1992; Shank et al., 1994), the genetically seizure-prone DBA/2 mouse (Nakamura et al., 1994), the spontaneously epileptic rat (Nakamura et al., 1994) and the amygdala-kindled rat (Kimishima et al., 1992; Wauquier et al., 1996). However, there are no data of its use in terminating SE in either animal models or clinically in patients, although there is an anecdotal report of its successful use in a patient refractory to various AEDs. The present study is the first to evaluate TPM in a refractory model of SE and the results are not very encouraging. Nevertheless, its efficacy in less severe models of SE needs to be ascertained. Interestingly a recent report suggested that when combined with the NMDA antagonist budipine, TPM is capable of terminating SE at a relatively low dose (20 mg/kg) and in the same SE model as that used in the present study (Fisher et al., 2002).

That TGB is effective in terminating refractory SE, as observed in the present study, is encouraging and confirming similar data reported by Walton et al (1994) using the homocysteine thiolactone model. TGB is also able to prevent SE and reduce seizure severity when administered before SE induction (Halonen et al., 1996). Taken together, these data would suggest that TGB maybe a useful drug in the treatment of SE and further studies, both experimental and clinical, are warranted.
In summary, the present study showed that high dose FosPHT (100 mg/kg) was effective in reducing seizure severity but not in aborting refractory SE; whereas high dose TPM (160 mg/kg) was completely ineffective in reducing seizure severity or aborting SE. In contrast, TGB (40 mg/kg) aborted refractory SE completely and quickly in a dose-dependent manner.
7 STUDY OF THE NEUROPROTECTIVE EFFECT OF FOSPHENYTOIN, TIAGABINE AND TOPIRAMATE IN THE MODEL OF REFRACTORY STATUS EPILEPTICUS

7.1 Introduction

Prolonged seizures are associated with injury to vulnerable neurons. Following SE a specific pattern of neuronal cell loss has been documented both in humans (DeGiorgio et al., 1992; Leifer et al., 1991) and in various animal models (Wasterlain et al., 1993). The hippocampus is especially vulnerable with cell loss in the hilus, CA1 and CA3 regions, but relatively sparing the dentate granule cells and CA2 region (Sloviter, 1987; DeGiorgio et al., 1992). This is also the pattern of damage commonly seen in the hippocampi of patients with refractory TLE (Dam, 1980).

Identification of compounds that attenuate injury after prolonged seizures could be of value in the management of refractory SE. Numerous studies have been carried out to explore the neuroprotective effect of AEDs and putative chemical agents in different animal SE models. MK-801, a non-competitive NMDA receptor antagonist, when administered both before and after SE induction, is observed to reduce hippocampal neuronal loss in SE models induced by pilocarpine, kainate injection or perforant path stimulation (MacGregor et al., 1997; Rice et al., 1998; Halonen et al., 1999). DZP, VPA, pentobarbital, clonazepam and ketamine (another non-competitive NMDA receptor antagonist), have been reported to have protective effects against seizure-induced brain damage and spatial memory deficits in a SE model induced by pilocarpine injection (Turski et al., 1987; Lemos et al., 1995; Hort et al., 1999).
Vigabatrin, felbamate and gabapentin administered before or after kainate treatment, decreased neuronal damage in the hippocampal CA3a and CA1 (Chronopoulos et al., 1993; Halonen et al., 1995; Cilio et al., 2001). CBZ treatment prior to SE induction in the perforant path stimulation model reduced hippocampal neuronal loss and deficit in spatial memory (Halonen et al., 1999; Kelsey et al., 2000). Halothane used as an anaesthetic agent during SE induction has also been reported to prevent neuronal loss in hilus, CA1, CA3 (Walker et al., 1999).

TGB, by enhancing GABA mediated neuronal inhibition, was reported to reduce hippocampal CA1 and CA3 pyramidal cell loss when administered before the induction of SE (Halonen et al., 1996). TGB has also been reported to reduce the brain infarction area and necrosis in the hippocampus CA1 pyramidal cell layer in ischaemic rats (Inglefield et al., 1995; Yang et al., 2000).

Single dose TPM administration after SE attenuated seizure-induced hippocampal neuronal injury (Niebauer et al., 1999). Chronic TPM administration following SE improved cognitive function but did not prevent neuronal loss in the hippocampus of developing brains (Cha et al., 2002). TPM has also been reported to enhance the recovery of facial nerve function after injury by enhancing neurite outgrowth (Smith-Swintosky et al., 2001).

PHT is reported to reduce neuronal loss and offer protection over neuronal function in hippocampal slices and neocortical cultures simulating the in vivo ischaemic environment, and in the model of compressive spinal cord injury (Weber et al., 1994; Boehm et al., 1994; Probert et al., 1997; Schwartz et al., 2001). PHT also preserved retinal ganglion cells after partial optic nerve crush (Naskar et al., 2002). However, its
efficacy in protecting the neuronal injury in the experimental SE model induced by pilocarpine injection was rather disappointing (Turski et al., 1987). FosPHT has also been reported to completely prevent hippocampal CA1 neuronal loss in a global ischaemia model (Chan et al., 1998). However, there have been no reports of the neuroprotective properties of FosPHT in SE models.

The present study evaluated and compared the neuroprotective effect of TGB, TPM and FosPHT in a model of refractory SE induced by perforant path stimulation.

7.2 Methods

Rats used in this study were from those used in the study of anticonvulsant efficacy of FosPHT, TGB and TPM in Chapter 6. The methodology used is described in Chapter 6. Fourteen days after SSSE induction and the evaluation of AED efficacy, rat brains were prepared for histological study as described in section 2.9.

7.3 Histology with vehicle treatment

The histology of hippocampus and CA1 of a vehicle (20% DMSO in saline) treated rat is shown in Figure 7.1. A moderate to severe degree (approximately 50%) of neuronal cell loss was observed in CA1, CA3 and hilus regions. Neuronal density in the stimulated side of the brain was 8.2 ± 0.39 in CA1, 7.18 ± 0.64 in CA3 and 3.54 ± 0.59 in hilus. These values were similar to those of the contralateral side of the brain (P > 0.05, Mann-Whitney U test). Compared to unstimulated rats and saline treated SSSE
Figure 7.1. Hippocampal histology (× 2.5) and close up of CA1 (× 40) from animals in SSSE treated with vehicle, FosPHT 100 mg/kg, TGB 40 mg/kg and TPM 160 mg/kg.
rats, the neuronal density in vehicle treated rats was significantly lower than the former
(P < 0.01, Mann-Whitney U test), but comparable to the latter (P > 0.05, Mann-Whitney
U test) in hippocampal CA1, hilus and CA3 regions (Figure 7.2). These data suggest that
the vehicle did not prevent neuronal loss.

7.4 Histology with drug treatment

The histology of hippocampus and CA1 of rats treated with FosPHT, TGB
and TPM is shown in Figure 7.1. Under a high-power microscope (x 100), neuronal loss
in the hippocampal CA1, CA3 and hilus regions was approximately 40-50% in all drug-
treated animals. Table 7.1 shows the mean neuronal density in these brain regions after
FosPHT, TGB and TPM treatment. In animals treated with FosPHT, there was no
difference between the stimulated and contralateral sides (P > 0.05, Mann-Whitney U
test). Furthermore, when the neuronal density of FosPHT group and that of vehicle
group were compared, no statistical difference was observed (P > 0.05, Mann-Whitney
U test) (Figure 7.3). However, a significant difference was observed between
unstimulated control animals and FosPHT treated animals (P < 0.01, Mann-Whitney U
test), suggesting that FosPHT did not stop the neuronal loss after refractory SE. This
result pattern was also seen in TGB and TPM treated rats (Figure 7.3).
Figure 7.2. Neuronal density in hippocampal CA1, CA3 and hilus in control, saline treated and vehicle treated groups. The neuronal density is the number of neurons in each counting box (20µm x 20µm x 5µm). Values are mean ± SEM, n = 5. The neuronal density was significantly lower in saline and vehicle treated SSSE rats than that in unstimulated control rats in all three hippocampal regions (P < 0.01, Mann-Whitney U test).

Figure 7.3. Neuronal density in hippocampal CA1, CA3 and hilus in FosPHT, TGB, TPM treated groups, compared to vehicle treated group. The neuronal density is the number of neurons in each counting box (20µm x 20µm x 5µm). Values are mean ± SEM, n = 5. There was no difference in neuronal density between vehicle and any of the drug treated group in all three hippocampal regions (P > 0.05, Mann-Whitney U test).
Table 7.1. Neuronal density in hippocampal CA1, CA3 and hilus after 100 mg/kg FosPHT, 40 mg/kg TGB, 160 mg/kg TPM and vehicle administration in SSSE rats. The neuronal density is the number of neurons in each counting box (20μm x 20μm x 5μm). Values are mean ± SEM, n = 5.

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7.5 Discussion

The principal observations from the present study were (1) SSSE is associated with approximately 50 % neuronal loss in hippocampal CA1, CA3 and hilus; (2) TGB, TPM and FosPHT did not reduce neuronal loss in hippocampal CA1, CA3 and hilus and therefore had no neuroprotective effect in this model.

The perforant path stimulation model producing limbic SE has the advantage that any effects seen can be truly attributed to seizure activity and not to extrinsic chemoconvulsants. In addition, as the model involves predominantly focal clinical manifestations, hypoxia or ischaemia from vigorous body movement are unlikely to be contributory factors in neuronal damage and cell loss. Indeed Pinard’s study indicated
that in the limbic SE model, concurrent increase in blood flow more than met the increase in oxygen demand (Pinard et al., 1984).

That FosPHT did not prevent hippocampal neuronal loss, in the present study, is in line with that of PHT in pilocarpine induced SE model (Turski et al., 1987). However, these data are in contrast to other reports whereby PHT was neuroprotectant after ischaemia at lower doses (5-100 μM) (Weber et al., 1994; Boehm et al., 1994; Probert et al., 1997). TGB at doses of 50-200 mg/kg prevented hippocampal neuronal damage in a similar perforant path stimulation induced SE model (Halonen et al., 1996). However, there are some methodological differences between the study of Halonen et al (1996) and the present study. Firstly, in Halonen’s study, perforant path stimulation duration was much shorter, which greatly reduced SE severity, since the refractoriness of SE is a function of the length of stimulation duration (Mazarati et al., 1998). Secondly, as TGB was administered prior to SE induction, the reported neuroprotective effect may be the result of reduced severity of seizures and SE. With regard to TPM, the lack of a neuroprotective effect, observed in the present study, was similarly observed in a study of developing rats at 20 days of age with neonatal seizures and SE (Cha et al., 2002). In contrast TPM (20-80 mg/kg) was associated with neuroprotective property in adult rats with limbic seizures induced by dorsal hippocampus electrical stimulation (Niebauer et al., 1999). However, this model was not self-sustaining and refractory since seizures stopped once the stimulation was terminated. These data raise the possibility that the neuroprotective property of TPM is a model-specific feature, which may largely depend on the severity of seizures and SE and therefore the degree of neuronal damage by the time of drug administration. This is probably supported by the fact that in this particular SE model induced by 120-minute perforant path stimulation, there has been no
report of neuroprotective agent, except halothane (Walker et al., 1999), which was given throughout the process of SE induction and as a result probably reduced the severity of seizures and thus the damaging effect on neurons.

Many factors are involved in selective neuronal damage in SE: excitotoxicity (Meldrum, 1991; Routbort et al., 1999; Tuunanen et al., 1999); calcium influx into cell (Stout et al., 1998), activation of apoptotic pathways (Ankarcrona et al., 1995), oxidative damage via the production of free radicals (Beal, 1996), nitric oxide production (Almeida et al., 1998) and mitochondrial dysfunction (Nicholls et al., 1999; Cook et al., 2002). In particular, burst discharge in SE is responsible for selective neuronal damage in hilus, CA1, CA3 (Meldrum 1974; Olney, 1983; Sloviter 1983, 1996). In SE, regardless of the seizure-inducing agent, there is massive calcium loading of mitochondria focally in dendritic fields of CA1, CA3 after 60 to 120 minutes of seizure activity. The grossly swollen calcium loaded mitochondria are visible after 30 minutes of burst discharge. These changes are reversible over 30 to 60 minutes if seizure-like discharge is stopped (Evans et al., 1984; Griffiths et al., 1984). The critical duration of seizure activity for inducing ischaemic cell damage lies between 82 and 120 min (Meldrum, 1973a). Mitochondrial poisoning by calcium overload is the critical link between the burst discharge and ischaemic cell damage (Meldrum, 2002). Subsequently, classical patterns of selective neuronal loss, phagocytosis and gliosis in CA1, CA3 and hilus appeared by 7 to 21 days after surviving from SE (Meldrum et al., 1972).

Meldrum's findings clearly indicate that the burst discharge needs to be sustained for 80-120 minutes to cause permanent neuronal loss, whereas 30-60 minutes of burst discharge leads to reversible neuronal damage. Less stress may only produce apoptosis or immediate early gene expression with enhanced expression of many
enzymes and receptor subunits. The oxidative stress cascade leading to final cell damage and neuronal loss following SE can be greatly alleviated consequent to the shortening of stimulation duration. This is supported by the fact that DZP applied at 60 minutes of seizure duration reversed the appearance of highly swollen, calcium-loaded mitochondria (Evans et al., 1984; Griffiths et al., 1984). In the present study the hippocampal neuronal damage might have already become irreversible by the time 120-minute electrical stimulation was terminated.

In summary, the present study confirmed previous reports that SE resulted in a specific pattern of neuronal damage in the hippocampus, and that in this refractory model of SE TGB, FosPHT and TPM offered no protection against neuronal damage.
8 GENERAL DISCUSSION

SE is considered to be one of the most severe forms of epilepsy with significant morbidity and mortality. Existing drugs used in the management of SE are not the most ideal in terms of their anticonvulsant efficacy and accompanying side effects. There is therefore a significant need for new drug treatments and this thesis sought to investigate some of the new AEDs in relation to their pharmacokinetic and neuropharmacokinetic inter-relationship, and their efficacy and neuroprotective effects in an animal model of SE.

Methodological Considerations

In order for the optimal therapeutic potential of a drug to be achieved, it is essential to devise a rational dosing regimen based on its pharmacokinetic characteristics. Pharmacokinetic considerations are important both for deciding the initial dosage and the dosing interval, and also in relation to ascertaining whether therapeutic drug monitoring could be useful as an aid to optimising treatment. For centrally acting drugs such as AEDs, it is important to establish the relationship between blood concentrations and concentrations in the CNS (CSF and brain ECF). However, these inter-relationships have been difficult to ascertain in humans, partly because of limited accessibility of CSF and the availability of only single-point determination of CSF. Furthermore, whole brain (post-mortem or surgically excised brain tissue) analysis has been limiting because of paucity of samples and also because such analysis may not actually reflect events at the extracellular level of drug action. Although recently the technique of microdialysis has allowed some studies of AEDs in humans (Lindberger et
al., 1999; Scheyer et al., 1994b), these data are rather limited. Consequently, more extensive and detailed pharmacokinetic and neuropharmacokinetic data can only be obtained in animal models and this thesis used such a model (freely behaving and freely moving) to study PHT, FosPHT and TGB.

With regard to animal models of SE, a severe refractory model was used to evaluate the antiepileptic potency and neuroprotective effects of FosPHT, TGB and TPM. The consequence of this is that drugs that are not effective in this particular model may be effective in less severe models of SE.

**Correlation of the pharmacokinetics and neuropharmacokinetics of FosPHT and PHT with anticonvulsant efficacy in the SE model**

As a PHT pro-drug, the physiochemical properties of FosPHT (solubility, pH, irritation to local soft tissue and blood vessels, time needed for conversion to PHT) differ significantly from that of PHT. While the pharmacokinetic and neuropharmacokinetic characteristics of PHT have been well defined both in animals and in man (Browne et al., 1989b; Hooper et al., 1973; Lunde et al., 1970; Scheyer et al, 1994a; Lolin et al., 1994; Walker et al., 1996), the extent to which different physiochemical properties of FosPHT would affect the pharmacokinetics and neuropharmacokinetics of PHT derived from FosPHT at the site of drug action has not previously been investigated. Indeed there has only been one study of PHT pharmacokinetics derived from FosPHT, and this entailed rat whole brain homogenates (Walton et al., 1999), which do not necessarily reflect PHT concentrations at the site of drug action.
Some pharmacokinetic features of AEDs are particularly important in the treatment of SE: (1) \( T_{\text{max}} \) in the brain which, limited by the rate of blood flow to the brain and the rate of penetration through BBB, determines the rate of the drug reaching its maximal therapeutic effect and therefore, the value of the drug in the treatment of SE. A rapid \( T_{\text{max}} \) in the brain is critical for a drug to be deployed in the early stage of SE; (2) the ratio of drug in the brain (CSF or ECF) compartment to that in the blood compartment. This is dependent on the drug’s physiochemical properties, the transport mechanisms through BBB and drug affinity to the brain tissue component, and affects drug dosage needed for effective treatment; (3) the ability to achieve rapid equilibration of drug concentrations between blood and brain compartments. At equilibration, drug concentrations in the brain compartment could be readily predicted by the therapeutic drug monitoring in the blood and the drug dosing-interval can be determined on the basis of the \( t^{1/2} \) in the blood compartment; (4) differences in brain regional distribution of the drug. This is important as certain types of SE originate from different but specific parts of the brain.

Data presented in this thesis suggest that FosPHT can be suitably deployed in the early stage of SE. PHT penetrated BBB and BCB readily and \( T_{\text{max}} \) was reached by approximately 12 minutes in the CSF compartment and by 30 minutes in the ECF compartment after FosPHT IV administration. These pharmacokinetic characteristics are in line with the observation in chapter 6 that during SE, EEG spike frequency and amplitude started to decrease at the time of first sampling (10 minutes after FosPHT IP administration) and reached their maximal reduction at the time of second sampling (20 minutes after FosPHT IP administration). Furthermore, PHT derived from FosPHT is
associated with long-lasting anticonvulsant efficacy in the treatment of SE, as evidenced by the finding that the reduction in EEG spike amplitude and frequency in the refractory model of SE lasted > 3 hours. This feature is in line with the neuropharmacokinetic data from the present study that after FosPHT administration PHT t½ in the brain ECF was approximately 4 hours (Wang et al., 2003).

In this thesis the pharmacokinetic and neuropharmacokinetic parameters of PHT after PHT administration were found to be comparable to those after FosPHT administration. It was further supported by the fact that the anticonvulsant potency of PHT (Holtkamp et al., 2001) and FosPHT (in the present study) in the refractory model of SE is comparable. The consistency between the pharmacokinetic data of PHT after either PHT or FosPHT administration and their efficacy in the SE model corroborate the validity of studies described in this thesis and the importance of studying drug pharmacokinetics and neuropharmacokinetics in the management of SE and in the prediction of anticonvulsant efficacy of AEDs.

Correlation of the pharmacokinetics and neuropharmacokinetics of TGB with anticonvulsant efficacy in the SE model

The pharmacokinetic and neuropharmacokinetic data of TGB presented in this thesis suggest that TGB may be associated with numerous advantageous characteristics in the treatment of SE: (1) rapid BBB and BCB penetration and short Tmax. After IP administration TGB was detectable in the CSF and brain ECF compartments at the time of first sampling (10 minutes), and Cmax was reached at approximately 30 minutes post dose. The time lag between Tmax in the blood and in the
brain (the CSF and ECF compartments) was only 15 minutes. These pharmacokinetic features are in line with the anticonvulsant efficacy of TGB in that EEG spike frequency and amplitude in SSSE rats declined significantly at the first sampling time (10 minutes) after TGB administration, and continued to decline until 40 minutes post dose when all the EEG spikes were suppressed. These features suggest that TGB would be a suitable drug for the treatment of SE at the initial stage; (2) slow elimination from the brain and relatively long $t_{1/2}$ (> 2 hours) in the brain ECF compartment. These characteristics suggested that TGB would maintain its efficacy for a reasonable length of time in the treatment of SE and would not require frequent top-up dosing, as is needed for DZP. Indeed in this thesis it was observed that after TGB administration to rats in SE the behavioural and electrographic activity were suppressed and the anticonvulsant effect was maintained throughout the 3-hour post-dose period.

The relationship of TGB administration with non-convulsive status epilepticus / encephalopathy

Since Walton (1994) first reported high amplitude 3-5 Hz spike-wave activities in rats (termed encephalopathy) associated with TGB administration, there have been many reports about TGB inducing non-convulsive status epilepticus in human subjects (Schapel et al., 1996; Eckardt et al., 1998; Balslev et al., 2000; Piccinelli et al., 2000; Skodda et al., 2001; Fitzek et al., 2001; Zhu et al., 2002). The phenomenon observed in animal studies and in humans was considered comparable and attributed to the same mechanisms. It was suggested that GABAergic drugs might be epileptogenic in patients with epilepsy (Schapel et al., 1996) and that non-convulsive status epilepticus
following TGB treatment was the result of GABAergic hyperfunction in the brain (Solomon et al., 1998). In the present study continuous slow activities were observed in rats’ EEG at 2 hours after 40 mg/kg TGB administration without accompanying EEG spikes or behavioural manifestations. After 20 mg/kg TGB administration, slow activities in EEG were visible for only a short period of time, as later on the EEG was marked by continuous high amplitude spikes. This phenomenon however, should be interpreted with caution as the anticonvulsant potency of TGB was not affected, and there were no EEG spikes or accompanying behavioural manifestations in the rats administered 40 mg/kg TGB. The exact mechanisms are not clear. Whether these slow activities were just the post-ictal events in the rats or the result of GABA hyperfunction is not known, as in the clinical setting non-convulsive status epilepticus is reported in only a fraction of patients receiving TGB treatment. Nevertheless, given the slow elimination of TGB from the brain, as indicated by the data presented in this thesis that TGB $t^{1/2}$ was three times longer in the brain ECF compartment than that in the blood compartment, the theory of GABA hyperfunction after TGB administration is a possible mechanism to consider.

Correlation of the neuropharmacokinetics of TPM with anticonvulsant efficacy in SE model

With regard to TPM, although there has been no data of the neuropharmacokinetics in the brain ECF compartment, studies in the CSF compartment in patients showed high CSF / plasma TPM concentration ratios (0.81-0.85) (Lindberger et al., 1999; Christensen et al., 2001). In addition, with its effect in various seizure
models (Nakamura et al. 1994; Shank et al., 1994; Wauquier et al., 1996) and in patients with various seizure types (Sachdeo et al., 1997; Biton et al., 1999), the neuropharmacokinetic features seem to be unlikely to hinder its action in the SE model. Nevertheless, TPM was ineffective in the perforant path stimulation induced SE model and would suggest that its mechanism of action is not useful in the control of SE.

Further thoughts about the efficacy of FosPHT, TGB and TPM in the refractory model of SE and their underlying mechanisms

In general, the rational design of AEDs is directed towards drugs that enhance GABAergic neurotransmission and/or attenuate glutamatergic neurotransmission (Rogawski et al., 1990; Loscher et al., 1994). Another potential strategy for termination of the development of epileptic activity is through the pharmacological modification of Na⁺, K⁺, and Ca²⁺ channel conduction (Rogawski et al., 1990). The AEDs investigated in the present study (TGB, FosPHT and TPM) have different molecular structures and differ in the mechanisms of action that underlie their antiepileptic effects.

In the management of SE, especially in the early stage, suitable pharmacokinetic and neuropharmacokinetic features are as important as the underlying anticonvulsant mechanisms that are determined by the chemical structure of AEDs in planning treatment regimens and optimising treatment outcome. In the present study while TGB dose-dependently aborted SE in the refractory model used, FosPHT and TPM at higher doses failed to do so. It is necessary to consider factors both in the
pharmacokinetic and neuropharmacokinetic aspects, as well as in the aspects of chemical structures of these drugs.

From the point of view of antiepileptic mechanisms, FosPHT is a pro-drug of PHT, which is a voltage-dependent Na\(^+\) channel blocker; while TGB inhibits GABA uptake into neurons and glial cells in the brain so as to increase GABA content in brain synaptic clefts. TPM has multiple antiepileptic mechanisms: antagonising glutamate excitatory transmission via the AMPA receptor; blocking the voltage-dependent Na\(^+\) channel and inhibiting carbonic anhydrase activity. The different anticonvulsant efficacy of FosPHT, TGB and TPM observed in the present study suggests that, in the case of refractory SE, drugs that enhance GABA-mediated neuronal inhibition maybe best suited. Whereas functional antagonists of Na\(^+\) channel or antagonists of glutamate-mediated neuronal excitation via the AMPA receptor may generally be ineffective. Indeed similar conclusions were reported in a cocaine-induced seizure model in mice, in which various AEDs and other putative agents were tested (Gasior et al., 1999). Gasior and colleagues (1999) found that clobazam, flunarizine, lamotrigine, TPM, and zonisamide were ineffective against seizures up to doses producing significant motor impairment. In contrast, felbamate, gabapentin, loreclezole, losigamone, progabide, remacemide, stiripentol, TGB, and vigabatrin produced dose-dependent protection against cocaine-induced convulsions.

*The neuroprotective effects of FosPHT, TGB and TPM in the refractory model of SE*

The investigation of the neuroprotective effects of FosPHT, TGB and TPM in this thesis yielded rather disappointing results in that none of these AEDs was
associated with neuroprotection in the hippocampal CA1, CA3 and hilus region, although TGB terminated SSSE rapidly and completely while FosPHT reduced the electrographic and behavioural seizure activity by 50%. There has been only one published report that halothane reduce neuronal damage (Walker et al., 1999). However, as halothane was given during the process of perforant path stimulation, the severity of neuronal damage may have been reduced by the early administration, which is not comparable to the present study protocol. The lack of neuroprotective effects in this perforant path model perhaps is not so surprising given that the model used in this thesis is particularly refractory (with 120 minutes continuous electrical stimulation and resultant long duration of seizures). As highlighted by Meldrum (1972, 1973a) that burst discharges longer than 80-120 minutes lead to irreversible neuronal damage, whereas the neuronal damage could be reversible by appropriate AED treatment if the burst discharges are of 30 - 60 minutes duration (Evans et al., 1984; Griffiths et al., 1984). Thus the lack of neuroprotective effects with all the AEDs studied in this thesis may possibly fit into Meldrum’s theory. Mitochondrial dysfunction may play an important role in the process of neuronal death after prolonged seizures in this model, with reduced brain glutathione levels and reduced brain aconitase and α-ketoglutarate dehydrogenase (part of the Krebs cycle in the mitochondrial matrix) activity in the early period after SSSE (Cock et al., 2002), and even after TPM administration (unpublished data by Andrew Fisher in our group). These results suggest that early treatment in the clinical management of SE is essential so as to prevent occurrence of permanent neuronal damage and chronic seizures.
Summary and further study

This thesis described various studies designed to ascertain pharmacokinetic and neuropharmacokinetic inter-relationship of various AEDs in a freely moving rat model and their anticonvulsant potency and neuroprotective effects in a refractory model of SE. Studies exploring the underlying mechanisms of excitatory and inhibitory neurotransmission in the course of SE induction and maintenance were also undertaken.

However, future study needs to extend the findings reported in this thesis: (1) in terms of the neuropharmacokinetics of FosPHT and TGB in the brain, other brain regions apart from the frontal cortex and hippocampus should be investigated so as to establish the exact drug distribution in the brain ECF compartment; (2) the SE model used in this thesis was a refractory model and none of FosPHT, PHT and TPM was effective. Future studies should use a perforant path stimulation protocol that produces less refractory SE so as to ascertain the antiepileptic efficacy of these AEDs; (3) in the study of ECF amino acid neurotransmitters during SE induction and maintenance, the sample size was small (n = 3) and needs to be expanded. Furthermore, the techniques should be fine tuned so as to allow correlation of each single seizure with the concurrent amino acid concentration change. This would allow a more detailed investigation into the mechanisms of SE induction and maintenance in this refractory model of SE; (4) since there has been no report of the pharmacokinetics and the concurrent neuropharmacokinetics of TPM in the blood, CSF and brain ECF, this should be studied so as to establish the pattern how TPM exerts its action in the brain and the inter-relationship of TPM kinetics in the blood and brain compartments.
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249


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253


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