Chapter I

Introduction

1. Epilepsy

Epilepsy is one of the most common diseases of the brain, affecting at least 50 million persons worldwide (McNamara, 2001; Löscher, 2002). Epilepsy is a chronic and often progressive disorder characterized by the periodic and unpredictable occurrence of epileptic seizures which are caused by an abnormal discharge of cerebral neurons. Many different types of seizures can be identified on the basis of their clinical phenomena. These clinical characteristics, along with their electroencephalographic EEG features, can be used to categorize seizures. Seizures are fundamentally divided into two major groups: partial and generalized. Partial focal, local seizures are those in which clinical or electrographic evidence exists to suggest that the attacks have a localized onset in the brain, usually in a portion of one hemisphere, while generalized seizures are those in which evidence for a localized onset is lacking. Partial seizures are further subdivided into simple partial, complex partial and partial seizures evolving to secondarily generalized seizures, while generalized seizures are categorized into absence non convulsive, myoclonic, clonic, tonic, tonic–clonic and atonic seizures. In addition to classifying the seizures that occur in patients with epilepsy, patients are classified into appropriate types of epilepsy or epileptic syndromes characterized by different seizure types, etiologies, ages of onset and EEG features. More than 40 distinct epileptic syndromes have been identified, making epilepsy a remarkably diverse collection of disorders. The first major division of epilepsy are localization-related focal, local, partial epilepsies, which account for roughly 60% of all epilepsies, and generalized epilepsies, which account for approximately 40% of all epilepsies. An epilepsy or epileptic syndrome is either idiopathic, which is virtually synonymous with genetic epilepsy, or symptomatic, i.e. due to structural lesion or major identifiable metabolic derangements. Both type of seizure and epilepsy determine the

choice and prognosis of therapy. For instance, the most common and most difficult-to-treat type of seizures in adult patients are complex partial seizures, while primary generalized tonic–clonic 'grand mal' seizures respond in most patients to treatment with anticonvulsants. For many of the seizure types and epilepsy syndromes there is little information about the pathophysiological basis. Insight into how partial seizures, generalized tonic–clonic seizures and generalized absence seizures arise is substantial, which is fortunate since these constitute around 90% of seizures (Rinaldi et al., 2000).

In the absence of a specific etiological understanding in any of the epilepsies or epileptic syndromes, approaches to drug therapy of epilepsy must necessarily be directed at the control of symptoms, i.e. the suppression of seizures. In fact, all currently available drugs are anticonvulsant rather than antiepileptic. The latter term should only be used for drugs which prevent or treat epilepsy and not solely its symptoms. The goal of therapy with an anticonvulsant drug is to keep the patient free of seizures without interfering with normal brain function. The selection of an anticonvulsant drug is based primarily on its efficacy for specific types of seizures and epilepsy (Mattson; 1995). For instance, valproic acid is usually the drug of choice for the generalized idiopathic epilepsies, while carbamazepine and phenytoin show the best balance of seizure control with relatively few adverse effects for the treatment of partial epilepsy (Mattson, 1995). In most patients with epilepsy the prognosis for seizure control is very good. However, a significant proportion of individuals with epilepsy suffer from intractable, i.e. pharmacotherapy resistant epilepsy despite early treatment and an optimal dosage of an adequate anticonvulsant drug (Forsgren; 1995). Thus, there is a clear need for new drugs or new strategies of therapeutic management. Although surgical treatment of epilepsy may be an alternative if anticonvulsant drugs fail, surgery for epilepsy might not be needed if we knew more about ways to prevent medical intractability or if we had more effective and less toxic anticonvulsant drugs (Löscher, 2002).

In addition to the need for new drugs for epileptic patients whose seizures are resistant to available anticonvulsants, new drugs with benefits in terms of side effects

and tolerability are needed even if they do not demonstrate greater efficacy than established anticonvulsants (Brodie and Dichter, 1996; Stringer, 1998; Löscher, 2002). Furthermore, in view of the fact that the therapeutic effectiveness of the older anticonvulsant drugs has usually been limited by their narrow therapeutic ratio, i.e. the ratio of toxic dose against the effective dose, it is hoped that an improved therapeutic ratio may be seen with some of the novel compounds currently being developed.

2. Drugs for treatment in epilepsy and mechanism of action

The serendipitous discovery of phenobarbital (PB) in 1912 marked the beginning of the modern pharmacotherapy of epilepsy (Brodie and Dichter, 1996). In the ensuing 70 years, phenytoin (PHT), carbamazepine (CBZ), ethosuximide (ESM), sodium valproate (VPA), phenobarbital (PB) and a range of benzodiazepines (BZDs) became available (Figure 1) and can be regarded as "established" antiepileptic drugs (AEDs; Brodie and Dichter, 1996). In the past decade, nine new agents (lamotrigine (LTG), oxcarbazepine (OXC), zonisamide (ZNS), vigabatrin (VGB), tiagabine (TGB), gabapentin (GBP), felbamate (FBM), topiramate (TPM) and levetiracetam (LEV)) have been licensed as add-on treatment for difficult-to control epilepsy (Table1) and still more are under evaluation (Dichter and Brodie, 1996). The undoubtedly beneficial expansion of the pharmacological armamentarium for the treatment of epilepsy does, however, complicate selection of the most suitable AED (or combination of AEDs) for individual patients. With limited clinical experience of the new agents, the mechanisms of action of individual AEDs may be an important criterion in this decision-making process (Brodie and Dichter, 1996).

Table 1 Proposed mechanisms of antiepileptic drug action

	Decrease Na^+ channels	Decrease Ca^{2+} channels	Increase K^+ channels	Increase inhibitory transmission	Decrease excitatory transmission
Established AEDs					
PHT	+++				
CBZ	+++				
ESM		+++			
PB		+		+++	+
BZDs				+++	
VPA	+	+		++	+
New AEDs					
LTG	+++	+			
OXC	+++	+	+		
ZNS	++	++			
VGB				+++	
TGB				+++	
GBP	+	+		++	
FBM	++	++		++	++
TPM	++	++		++	++
LEV		+		+	+

+++, Primary action; ++, Probable action and +, Possible action

Data from Meldrum (1996) and White (1999).

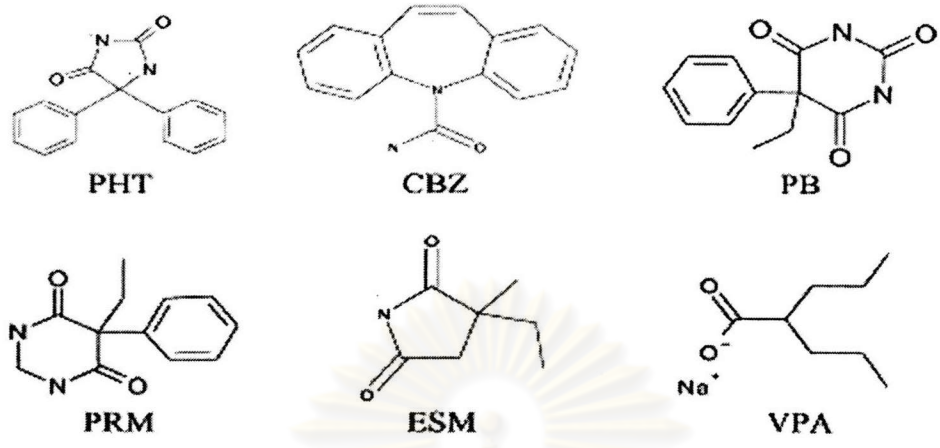

Figure 1 Molecular structure of the established antiepileptic drugs

2.1 Classification of mechanisms of action

Practical approaches to drug treatment have resorted primarily to symptom control, i.e., suppression of seizures. Although the mechanisms of action of the currently marketed AEDs are still not completely understood, they ultimately involve alteration of the balance between neuronal excitation and inhibition (White, 1999). At the cellular level, three basic mechanisms are recognized: modulation of voltage-dependent ion channels (Na^+, Ca^{2+}, K^+), enhancement of g-aminobutyric acid (GABA)-mediated inhibitory neurotransmission, and attenuation of excitatory (particularly glutamate-mediated transmission) (Meldrum, 1996; Soderpalm, 2002). It is, however, increasingly recognized that many AEDs possess multiple primary mechanisms. In addition, many less well-characterized mechanisms may also contribute to the anticonvulsant effect of any given compound. Finally, to complicate the issue further, the primary mode of action of some AEDs remains to be discovered.

2.2 Targets for antiepileptic drug action

2.2.1 Na^+ channels

In the nervous system, voltage-gated ion channels control the flow of cations across surface and internal cell membranes (Barchi, 1998). Of these, the Na^+ channel is arguably of principal importance. Voltage-dependent Na^+ channels are responsible for the upstroke of the neuronal action potential, and ultimately control the intrinsic excitability of the nervous system (Porter and Rogawski, 1992). The neuronal Na^+ channel has a multi-subunit structure that forms a Na^+-selective, voltage-gated pore through the plasma membrane. The protein structure undergoes conformational alterations in response to changes in membrane potential, regulating conductance through the intrinsic pore (Ragsdale and Avoli, 1998). The main structural component of the neuronal Na^+ channel is the a-subunit, which forms the ion-conducting pore and confers voltage dependency (Catterall, 1992). In the mammalian brain, the a-subunit associates with two auxiliary subunits designated b1 and b2. The b-subunits are not required for basic Na^+ channel activity, but they modulate the expression and function of individual channels (Ragsdale and Avoli, 1998). At normal membrane potentials, most Na^+ channels exist in a closed, resting state. Upon depolarization, the channel activates, facilitating ion flux. Thereafter, the Na^+ channel enters an inactivated state, from which it is not readily re-activated. Repolarization of the neuronal membrane rapidly converts the channel back to a resting state, from which it can respond to subsequent depolarization (Catterall, 1992; Ragsdale and Avoli, 1998). Neuronal Na^+ channels can cycle through these functional states within a few milliseconds. This characteristic is essential for sustaining the rapid bursts of action potentials necessary for some normal brain functions, and is implicated in the production of epileptic discharges. The neuronal Na^+ channel represents one of the

most important targets for AED action (Upton, 1994; Macdonald and Kelly, 1995; Meldrum, 1996; White, 1999; Soderpalm, 2002).

2.2.2 Ca^{2+} channels

Voltage-dependent Ca^{2+} channels share key structural elements and sequence homology with their Na^{+} channel counterparts (Barchi, 1998). The a1-subunit of the Ca^{2+} channel is the homologue of the a-subunit of the Na + channel. It forms the Ca^{2+}-sensitive channel pore and confers voltage dependency (Catterall, 1995). In the mammalian brain, the a1-subunit heterogeneously associates with other subunits designated b, g, and d. Voltage-sensitive Ca^{2+} channels can be broadly classified into low or high threshold, according to the membrane potential at which they are activated (Hofmann et al., 1994). The low-threshold T-type Ca^{2+} channel is expressed predominantly in thalamo-cortical relay neurons, where it is believed to be instrumental in the generation of the rhythmic 3-Hz spike-and-wave discharge that is characteristic of generalized absence seizures (Coulter et al., 1989). High threshold Ca^{2+} channels are subclassified by their pharmacological properties into L-, N-, P-, Q-, and R-types (Hofmann et al., 1994; Catterall, 1995; Dolphin, 1995). These channels are distributed throughout the nervous system on dendrites, cell bodies, and nerve terminals. The N-, P-, and Q-type channels, in particular, have been implicated in the control of neurotransmitter release at the synapse (Stefani et al., 1997). Interest in Ca^{2+} channels has heightened in recent years, following the identification of subunit-specific genetic mutations that can alter channel structure and/or function and that have been implicated in several human neurological diseases (Ophoff et al., 1998). Several AEDs have been reported to block voltage-sensitive Ca^{2+} channels in a subtype-specific manner, an effect that may contribute to their antiepileptic actions (Stefani et al., 1997).

2.2.3 K^+ channels

Neuronal K^+ channels are large protein complexes that form tetrameric structures, the monomers of which are structurally and genetically related to the a and a1-subunits of the Na^+ and Ca^{2+} channel, respectively (Barchi, 1998). The association of four subunits (monomers) in the neuronal membrane is required for the formation of a K^+ -sensitive pore and, therefore, channel function (Pongs, 1999). More than 40 distinct K^+ channel subunits have been identified, together with several auxiliary subunits. Given heterologous arrangement, it is possible that countless populations of K^+ channels, with individual functions and distributions, are expressed in the mammalian brain (Pongs, 1999).

At the neuronal level, K^+ channels are intimately involved in excitability. They are responsible for the action potential downstroke or, more specifically, repolarization of the plasma membrane in the aftermath of Na^+ channel activation (Pongs, 1999). Direct activation of voltage-dependent K^+ channels hyperpolarises the neuronal membrane and limits action potential firing (Porter and Rogawski,1992). Accordingly, K^+ channel activators have anticonvulsant effects in some experimental seizure models (Rostock et al., 1996), whereas K^+ channel blockers precipitate seizures (Yamaguchi and Rogawski, 1992). Potentiation of voltage-sensitive K^+ channel currents may prove to be an important target for future AED development. The novel antiepileptic agent retigabine, currently undergoing Phase II clinical trial, is believed to exert its effects, at least in part, by activation of the KCNQ2/ KCNQ3 K^+ channels (Rundfeldt and Netzer, 2000). Mutations in the KCNQ2/KCNQ3 channels have been reported in benign neonatal familial convulsions, a generalized epilepsy syndrome (Rogawski, 2000).

2.2.4 GABA-mediated inhibition

GABA is the predominant inhibitory neurotransmitter in the mammalian CNS, where it is released at up to 40% of all synapses (Olsen and Avoli, 1997). Impairment of GABA function is widely recognized to provoke seizures, whereas facilitation has an anticonvulsant effect (Löscher, 1999). GABA is synthesized from glutamate, exclusively in GABAergic neurons, by the action of the enzyme glutamic acid decarboxylase (GAD; Löscher, 1999). Upon synaptic release, GABA acts on its three specific receptors, $GABA_A$, $GABA_B$, and the newly characterized $GABA_C$. GABA receptors are distinguished by their pharmacology and function (Johnston, 1996). The $GABA_A$ receptor belongs to the ligand-gated ion channel superfamily, and responds to GABA binding by increasing Cl^- conductance, resulting in neuronal hyperpolarisation (Rabow et al., 1995). $GABA_B$ receptors are G-protein-linked (Olsen and Avoli, 1997). The activation of $GABA_B$ receptor, as a member of the G-protein-linked superfamily, decreases Ca^{++} and increases K^+ conductance in neuronal membranes. The effect on Ca^{++} conductance appears to be primarily associated with presynaptic P/Q and N-type Ca^{++} currents and modulation of K^+ conductance appears to be linked primarily with postsynaptic $GABA_B$ sites and with perhaps multiple types of K^+ channels. Decrease Ca^{++} conductance influences transmitter release and increases K^+ conductance associated neuronal hyperpolarization (Bowery, 2000; Bowery and Enna, 2000; Bowery et al., 2002). It has recently been proposed that $GABA_A$ and $GABA_B$ receptors may have evolved from the $GABA_C$ receptor, which is comparatively simpler in structure and pharmacology (Johnston, 1996).

Of the three receptor subtypes, the $GABA_A$ receptor, with its pentameric subunit array and central Cl^- ion pore, is perhaps the best understood (Rabow et al., 1995). $GABA_A$ receptors are composed of various combinations of 5 subunits, a (1 – 6), b(1 – 3), g(1 – 3), d, and e. Receptor physiology, pharmacology, and distribution differs,

depending on subunit composition (Mody, 1998). Theoretically, ten thousands of different $GABA_A$ receptors could exist, but naturally only 10 or fewer combinations of subtypes are encountered.

Following receptor activation, GABA is removed from the synaptic cleft into localized nerve terminals and glial cells, by specific membrane-bound transport molecules. Currently, four active transport systems, GABA transporter (GAT)-1, GAT-2, GAT-3, and betaine GAT (BGT)-1, have been described (Borden et al., 1992). GABA has a variable affinity for these transporters, and only GAT-1, predominantly located in the cerebral cortex and hippocampus, has GABA as its principal substrate (Guastella et al., 1990). After removal from the synapse, GABA is either recycled to the readily releasable neurotransmitter pool (GABAergic nerve terminals only) or metabolized (neurons and glial cells) to the inactive molecule succinic acid semialdehyde by the action of the mitochondrial enzyme GABA-transaminase (GABA-T; Meldrum, 1995).

Several AEDs exert their effects, at least in part, by actions on the GABAergic system. Increased GABA synthesis, increased release, allosteric receptor facilitation, and reduced inactivation have all been implicated in the mechanisms of action of commonly used agents (Sills et al.,1999). The GABA system also represents the most successful target for the rational design of novel antiepileptic compounds (Löscher, 1998).

2.2.5 Glutamate-mediated excitation

Glutamate is the principal excitatory neurotransmitter in the mammalian brain (Meldrum, 2000). Focal injection of glutamate induces seizures in animals, and over-activation of glutamatergic transmission or abnormal glutamate receptor properties are observed in certain experimental seizure models and human epilepsy syndromes (Meldrum, 1995). Inhibition of the neuronal release of glutamate and blockade of its receptor have received considerable attention in the search for novel AEDs (Meldrum, 2000).

Glutamate is synthesized from glutamine by the action of the enzyme glutaminase in glutamatergic neurons (Daikhin and Yudkoff, 2000). Following synaptic release, glutamate exerts its pharmacological effects on several receptors, classified into ionotropic and metabotropic families. Glutamate is removed from the synaptic cleft into nerve terminals and glial cells by the action of several specific transporters (Meldrum et al., 1999). Glial glutamate uptake is of principal importance. Glial cells convert glutamate into glutamine by the action of the enzyme glutamine synthetase. Glutamine is subsequently transferred to glutamatergic neurons, completing the cycle (Daikhin and Yudkoff, 2000).

Like GABA receptors, ionotropic glutamate receptors are comprised of various combinations of subunits forming tetrameric and pentameric arrays. They are classified into three specific subtypes, a-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA), kainate, and N-methyl-D aspartate (NMDA), which form ligand-gated ion channels, permeable to Na^{+} and, depending on subtype and subunit composition, Ca^{2+} ions (Trist, 2000). The NMDA receptor is further distinguished by having glycine as a co-agonist. The AMPA and kainate subtypes of the glutamate receptor are implicated in fast excitatory neurotransmission, whereas the NMDA receptor, quiescent at resting membrane potential, is recruited during periods of prolonged depolarization (Meldrum, 2000). The metabotropic family of glutamate receptors, also classified into three distinct subtypes (Groups I, II, and III), are G-protein linked and predominantly presynaptic, possibly controlling neurotransmitter release (Meldrum, 2000).

Although none of the commonly used AEDs exert their pharmacological effects solely by an action on the glutamate system, blockade of ionotropic glutamate receptors is believed to contribute to the antiepileptic activity of several compounds (Upton, 1994; Macdonald and Kelly, 1995; Meldrum, 1996; White, 1999). In addition, several AEDs have been reported to reduce glutamate release, although this effect may be more indicative of their actions on neuronal Ca^{2+} channels than a direct effect on the glutamate system (Stefani et al., 1997).

3. Strategies of antiepileptic drug development

There are at least three preclinical strategies which are used for development of new anticonvulsant drugs: (1) random screening of newly synthesized chemical compounds of diverse structural categories for anticonvulsant activity in animal models, (2) structural variation of known anticonvulsant drugs and (3) mechanism-based rational drug development, based on knowledge of the basic pathophysiological events involved in seizures or epilepsy (Löscher and Schmidt, 1994; Upton, 1994). All three strategies have generated clinically effective anticonvulsant drugs, although many scientists currently believe that the strategy of rational 'modern' drug development has important advantages over the more traditional other strategies.

Historically, all old anticonvulsant drugs have been found by serendipity, screening or structural variation of known drugs. Except the bromide and phenobarbital, the anticonvulsant effect of all standard anticonvulsant drugs was first determined in animal models, such as the maximal electroshock seizure or the pentylenetetrazole seizure tests, demonstrating that clinical activity can be predicted by such laboratory models (Löscher and Schmidt,1994). Therefore, seizure models in laboratory animals are still the most important prerequisite in preclinical search for new anticonvulsant drugs.

If one reviews the new clinically effective and introduced anticonvulsant drugs and groups them according to the existing preclinical strategy by which each drug has been developed, then screening or serendipity led to the development of felbamate, topiramate and lamotrigine, while oxcarbazepine and clobazam were developed by structural variation of known drugs (Löscher and Schmidt, 1994). Only three of the seven new, second generation anticonvulsants with proven clinical efficacy have been developed by mechanism-based rational development, namely vigabatrin, tiagabine and gabapentin (Löscher and Schmidt,1994). Various other novel 'third generation' anticonvulsant drugs are in preclinical or clinical development (Löscher and Schmidt, 1994; Bialer et al., 1996; White,

1997). All drugs with already demonstrated clinical activity e.g. levetiracetam, remacemide, fosphenytoin, dezinamide, losigamone have been found by screening or structural variation (Löscher and Schmidt, 1994). As with the old anticonvulsant drugs, the anticonvulsant effect of the novel clinically effective compounds was determined by seizure models in laboratory animals, substantiating that clinical activity can be predicted in this way.

The past decades have witnessed an increase in our knowledge on the pathophysiology of brain diseases and the basic mechanisms of drug activity that is without precedent (Dichter, 1994; McNamara, 1994). This knowledge generated several rational strategies for drug development, aimed to identify new anticonvulsant drugs with high specificity and selectivity of action. The most important strategies of rational design of anticonvulsant drugs have been (1) enhancement of GABA-mediated neuronal inhibition (2) diminution of glutamate-mediated neuronal excitation and (3) modulation of Na^+, K^+ and particularly Ca^{2+} ion channels (Löscher and Schmidt, 1994; Upton, 1994). All three targets for anticonvulsant drug development, i.e. GABAergic inhibition, glutamatergic excitation and intrinsic, voltage-dependent currents are thought to be critically involved in the pathophysiology of epileptic processes (Dichter, 1994).

3.1 Increase of GABAergic neurotransmission

The most successful of these rational strategies in terms of producing new clinically effective anticonvulsant drugs has been pharmacological enhancement of GABAergic neurotransmission. Of the various GABAmimetic drugs produced by this strategy, i.e. inhibitors of GABA aminotransferase such as vigabatrin, GABA uptake blockers such as tiagabine, GABA receptor agonists such as progabide and THIP (gaboxadol), and GABA receptor modulators, e.g. novel benzodiazepine receptor ligands such as partial agonists (e.g. bretazenil), subtype selective agonists (e.g. abecarnil) or neurosteroids, so far only vigabatrin and tiagabine proved to be effective in epileptic

patients. Both drugs, which act by increasing GABA levels in the synaptic cleft, are effective treatments for partial seizures in patients with drug resistance to the old drugs (Schmidt and Kramer, 1994; Chadwick, 1997). A third GABAmimetic drug with proven efficacy in partial epilepsy is gabapentin (Schmidt, 2002). Gabapentin has recently been shown to increase GABA turnover in some brain regions of laboratory animals (Löscher and Nolting, 1991), presumably by activation of glutamate decarboxylase (Taylor et al., 1992) and to enhance brain GABA levels in patients (Petroff et al., 1996) but in contrast to vigabatrin and tiagabine, gabapentin also exerts several other cellular action not related to GABA (Taylor, 1995).

Unfortunately, there are number of potential problems with increasing GABAergic neurotransmission as a strategy in anticonvulsant drug development. At least in laboratory animals, most GABAmimetic drugs produce tolerance and dependence (Löscher, 1986; Löscher and Schmidt,1994; Costa and Guidotti, 1996) which is well known from both experimental and clinical experience with barbiturates and traditional benzodiazepines. If drugs such as vigabatrin and tiagabine produce tolerance and dependence in patients has to await more clinical experience with these drugs, particularly in terms of monotherapy. Drugs which increase GABA concentrations, such as vigabatrin and tiagabine, are likely to aggravate non-convulsive absence, myoclonic seizures, most probable by an effect of enhanced GABA levels on GABA receptors (Löscher and Nolting, 1991). As a consequence, the spectrum of clinical efficacy of such compounds is relatively small, although vigabatrin is also effective against infantile spasms, i.e. in West syndrome (Löscher and Schmidt, 1994). Another potential disadvantage of some new GABAmimetic drugs, e.g. vigabatrin, is the induction of psychotic reactions (Loscher and Schmidt, 1994). Indeed, a hyperactive GABA system has been involved as a common etiological factor in both schizophrenia and affective psychosis (Schachter, 1995).

3.2 Decrease of glutamatergic neurotransmission

Another strategy, which has been used by several drug companies for development of anticonvulsant and neuroprotective compounds during recent years, is drug-induced decrease of glutamatergic neurotransmission (Löscher and Schmidt, 1994). Drugs developed by rational drug design include NMDA receptor antagonists, such as the noncompetitive antagonist MK-801 (dizocilpine) or the competitive antagonist, D-CPPene (3-(2-carboxypiperazin-4-yl)propenyl-1-phosphonate) and AMPA (a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor antagonists, such as NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo (F) quinoxaline) and GYKI 52466 (1-(4-aminophenyl) -4-methyl-7,8-methylendioxy-5H-2,3-benzodiazepine). As yet, clinical data are only available for NMDA receptor antagonists, including dextrorphan, an active metabolite of the antitussive dextromethorphan, which acts as a noncompetitive NMDA receptor antagonist (Löscher and Schmidt, 1994). In view of the increasing evidence that an abnormality of glutamate-mediated neurotransmission may critically contribute to the pathophysiology of seizures and epilepsy, development of glutamate receptor antagonists, particularly drugs blocking NMDA receptors, was thought to be one of the most promising strategies for rational anticonvulsant drug development (Dingledine et al., 1990; Löscher, 1993). However, both the clinical trials and more recent data from animal models demonstrated a number of severe problems associated with the mechanism (Loscher and Schmidt, 1994), so that further development of NMDA receptor antagonists for treatment of epilepsy has been subsided. The few clinical trials with MK-801, D-CPPene and dextrorphan did not yield any significant anticonvulsant effect of the drugs in patients with partial seizures (Löscher and Schmidt, 1994). Instead, treatment was associated with marked adverse effects, such as psychosis, impairment of learning and memory and impairment of motor function. This was unexpected because all these drugs had been well tolerated in phase I studies in healthy volunteers. Interestingly, several previous experimental observations indicated that limbic epileptogenesis enhances the adverse effect potential of NMDA receptor antagonists, leading to induction of proconvulsant effects, motor impairment and psychotomimetic

effects (Löscher and Honack, 1991; Löscher and Schmidt, 1994). Pre-existing susceptibility to focal seizures was suggested to be a prerequisite for proconvulsant effects of the noncompetitive NMDA receptor antagonist, ketamine, in rats (Löscher and Honack, 1990). The noncompetitive NMDA receptor antagonist, memantine, induced seizures in kindled rats at doses that were anticonvulsant in non-kindled rats (Löscher and Honack, 1990). Kindled rats were more sensitive than non-kindled rats to the induction of behavioral adverse effects (e.g. stereotypies) by competitive NMDA antagonists (Löscher and Honack, 1991). The glycine and NMDA receptor antagonist (+) -HA-966 ((+) -3-amino-1-hydroxy-pyrrolid-2-one) induced paroxysmal activity in limbic brain regions of kindled but not non-kindled rats (Wlaz et al. 1994) . The reasons for this functional difference between kindled and non-kindled rats remain to be elucidated (Wlaz et al., 1994) but all these data strongly suggest that models of chronic epilepsy, such as the kindling model, should be added to the preclinical assessment of adverse effects of NMDA receptor antagonists and probably also other investigational drugs in order to enhance the predictive value of such data for adverse effects occurring in patients with chronic brain disease (Wlaz et al. 1994).

It remains to be seen whether other strategies of glutamate antagonism, e.g. AMPA receptor antagonists or ligands for the metabotropic glutamate receptor provide advantages in terms of risk–benefit ratio for treatment of epilepsy. Drugs, such as lamotrigine or riluzole, which reduce glutamate release by blockade of Na^+ channels, clearly differ in their pharmacology from selective glutamate receptor antagonists and should not be assigned to this category. Recent in vitro experiments with other Na^+ channel blockers, i.e. carbamazepine and oxcarbazepine, have shown that these drugs, as lamotrigine, inhibit veratrine-induced glutamate release from rat brain slices at therapeutically relevant concentrations (Waldmeier et al., 1996). However, experiments in conscious rats with determination of veratrine-enhanced extracellular glutamate levels by microdialysis led to the conclusion that neither of these drugs in relevant doses inhibits physiological glutamate release *in vivo* (Waldmeier et al., 1996).

3.3 Modulation of ion channels

Similar to the glutamate directed strategy, the ion channel directed strategy of developing new anticonvulsant drugs failed to produce any clinically effective drugs with advantages over existing drug treatments. With respect to Na^+ channels, ralitoline was developed as a state dependent blocker of voltage-sensitive Na^+ channels, but both experimental and clinical studies failed to demonstrate any clear advantage over standard drugs, such as phenytoin and carbamazepine, so that further development was terminated (Löscher and Schmidt, 1994; Bialer et al., 1996). With respect to Ca^{2+} channels, several Ca^{2+} channel L-type blockers, such as verapamil, nifedipine, diltiazem and flunarizine have been clinically evaluated in epileptic patients, but only low or absent anticonvulsant efficacy was demonstrated by controlled trials (Löscher and Schmidt, 1994). However, as new antagonists for various Ca^{2+} channel subtypes are discovered, it remains possible that effective new anticonvulsants may emerge.

4. Valproic acid

Valproic acid (sodium valproate, VPA) is, at present, the most commonly used antiepileptic drug in the treatment of generalized epilepsy, and it is also effective in partial epilepsy. In spite of its wide use for many years, the mechanism of VPA action is still not fully understood. Its simple structure of a short-branched fatty acid (Figure 2) differs to a great extent from the substituted heterocyclic ring structures characterizing the traditionally used antiepileptic drugs. The antiepileptic action of VPA is probably due to a combination of several effects in the central nervous system (CNS) because of its wide spectrum of activity against different types of seizures and status epilepticus.

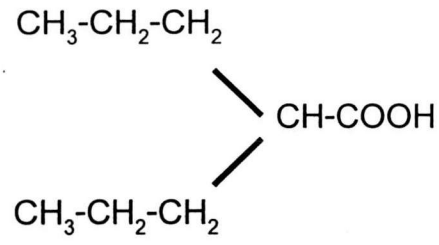

Figure 2 The structure of valproic acid (VPA)

4.1 Mechanisms of action of VPA

The antiepileptic properties of VPA were discovered serendipitously when VPA was employed in animal studies as a solvent for drugs under formal investigation. VPA has since proved to be an extremely useful AED, with a broad spectrum of activity and particular efficacy in the generalized epilepsies (Brodie and Dichter, 1996). However, the precise mechanisms by which it exerts its antiepileptic effects remain to be conclusively determined.

VPA has been reported to block voltage-dependent Na^+ channels. It reduces sustained repetitive firing of mouse neurons in culture (McLean and Macdonald, 1986) and reduces Na^+ currents in neocortical neurons (Zona and Avoli, 1990). However, rat hippocampal slice studies suggest that, unlike phenytoin and carbamazepine, VPA has no effect on the recovery of Na^+ channels from the inactivated state (Albus and Williamson, 1998).

VPA may also block T-type Ca^{2+} channels in a manner similar to that reported for ethosuximide. Such an effect would explain its efficacy against generalized absence seizures. However, the reduction of T-type Ca^{2+} currents observed with VPA in rat primary afferent neurons is modest and requires relatively high drug concentrations (Kelly et al., 1990). In addition, VPA appears to have no effect on Ca^{2+} channel conductance in rat thalamic neurons (Coulter et al., 1989).

There is evidence to suggest that VPA elevates whole brain GABA levels and potentiates GABA responses, possibly by enhancing GAD activity and inhibiting GABA degradation (Löscher, 1999). Some reports suggest that the drug also augments GABA release and blocks GABA uptake (Sills et al., 1996). The reproducibility of these effects has, however, been questioned (Rogawski and Porter, 1990). It is suggested that the GABAergic effects of VPA exhibit a degree of regional specificity within the brain and that inconsistent results reflect the resolution of individual studies (Rowley et al., 1995).

Electrophysiological recording techniques have reported that VPA selectively enhanced neuronal response to exogenously applied GABA (Rogawski and Porter, 1990; Davis et al., 1994). However, the concentrations required to potentiate GABA responses in the electrophysiology studies are far higher than the normal therapeutic levels, indicating that augmentation of GABA-mediated inhibition by the postsynaptic mechanism is unlikely to account for the anticonvulsant action of the drug under normal circumstances (Rogawski and Porter, 1990; Waterhouse and Delorenza, 1996).

VPA also reduced neurotransmission mediated by excitatory amino acid such as aspartic acid, glutamic acid and γ-hydroxybutyric acid (GHB), which produced absence-like seizures in animals (Chapman et al., 1982). In amygdaloid slices VPA suppressed the response mediated by NMDA receptors (Gean, 1994). However, glycine, which is an inhibitory neurotransmitter in brainstem and spinal cord, is not altered by VPA (Löscher, 1999).

4.2 Side effects and toxicity of VPA

The incidence of toxicity associated with the clinical use of VPA is remarkably low compared with other AEDs. The most common adverse effects are gastrointestinal disturbance, weight gain, thrombocytopenia, pancreatitis, neurological effects such as tremor or sedative and transient hair loss (Davis et al., 1994; Löscher, 1999; Greenwood, 2000). However, the drug is associated with two severe, albeit rare toxic

effects, fatal hepatotoxicity and teratogenicity (Brodie and Dichter, 1996; Davis et al., 1994; Löscher, 1999; Rowan, 1997).

Transient elevation of liver enzyme activity: alkaline phosphatase, glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT) have been observed in approximately 11% of patients receiving VPA (Jeavons, 1982). Fatal hepatotoxicity, occurred more frequently in patients less than 2 years old receiving polytherapy and did not appear to be related to the dose of VPA (Davis et al., 1994; Löscher, 1999). Studies in animals have shown that VPA exhibits teratogenic effects (Jeavons, 1982). In human, an estimated risk of 1 to 2% for neural tube defects, predominantly spina bifida aperta, with maternal use of VPA therapy has been reported (Bjerkedal et al., 1982; Davis et al., 1994; Dreifuss and Langer, 1988).

Despite the hepatotoxic and teratogenic effects of VPA in patients, this drug is considered a safe medication, provided certain precautions are dealt with, particularly in children less than 2 years of age and pregnant women (Löscher, 1999). Since VPA has two severe side effects, hepatotoxicity and teratogenicity, and less potent than other three established antiepileptic drugs; phenobarbital, phenytoin and carbamazepine, there is a substantially need to develop new derivatives of VPA with higher potency but lower toxicity (Bialer et al., 1994; Bialer, 1999).

5. N-Hydroxymethyl-2-propylpentamide (HPP)

HPP is an amine analogue of VPA which was synthesized in the laboratory of the Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University. The structure of HPP is shown in Figure 3. In preliminary studies, intraperitoneally administered HPP demonstrated a higher protection than VPA in both the maximal electroshock seizure (MES) and the pentylenetetrazole (PTZ) tests. However, HPP could not block the effect of bicuculline and was ineffective in strychnine test. Based on the relatively high medial lethal dose (LD_{50}), HPP possessed a same margin of safety

(LD_{50}/ED_{50}) as VPA (Supatchaipisit, 1995). In brain microdialysis studies, HPP significantly increased the level of cortical inhibitory neurotransmitter GABA in anesthetized rats (Supatchaipisit, 1995).

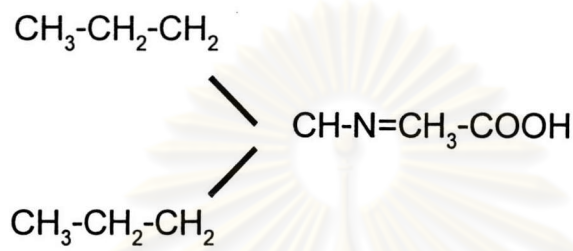

Figure 3 The structure of N-Hydroxymethyl-2-propylpentamide (HPP)

According to previous study in animal models, HPP has demonstrated a good prospect of being a potent broad spectrum antiepileptic drug with the same margin of safety as VPA. Extensive studies on its precise mechanism(s) of actions have to be accomplished before a definite conclusion could be reached.

6. Aims and objectives

The evidences presented above gave encouragement to extensive study on the precise mechanism of action of HPP, especially modulation of amino acid neurotransmission. Thus, the present study was aimed to determined:

1. effects of HPP on the levels of excitatory and inhibitory amino acid neurotransmitters in cerebral cortex of freely moving rats by using microdialysis technique,

2. effects of HPP on the $GABA_A$, glycine and NMDA currents in acutely dissociated rat hippocampal neurons by using the whole-cell patch clamp technique.