Chapter I

Introduction

Epilepsy

Epilepsy is a disorder of brain function characterized by recurrent episodes of neurological or behavioral manifestations, which commonly termed seizures caused by the abnormally synchronous and excessive discharge of large populations of neurons. It is therefore a chronic neurological condition with permanent pathophysiological features (Avoli, 1997; Ure and Perassolo, 2000).

Epilepsy includes a group of heterogeneous and diverse conditions. The terms seizure and epilepsy are not synonymous, and the distinction must be made clear. A seizure is an abnormal behavior (with symptoms or signs) resulting from abnormal, excessive, hypersynchronous discharges of cortical neurons. It is an observable phenomenon that is finite in time. Epilepsy refers to a chronic condition characterized by recurrent seizures (Stringer, 1998; Gilroy, 2000; Benbadis, 2001).

A syndrome is a cluster of symptoms and signs that occur together but, unlike a disease, do not have a single known cause or pathology. An epileptic syndrome (or epilepsy) is a constellation of symptoms (including seizures), signs, and other findings that occur together (Benbadis, 2001).

About 0.5% of the population suffers from epilepsy, with most patients having their first seizure before 18 years of age (Stringer, 1998; Ure and Perassolo, 2000). It is estimated that about 75 to 80 % of patients with epilepsy can have their seizures controlled with the drugs currently available (Craig, 1997; Porter and Meldrum, 1998).

All people are capable of experiencing seizures. Brain insults such as fever, hypoglycemia, hyponatremia, and extreme acidosis or alkalosis can trigger a seizure, but if the condition is corrected, seizures do not recur (Stringer, 1998). Epileptic seizures often

cause transient impairment of consciousness, leaving the individual at risk of bodily harm and often interfering with education and employment (McNamara, 1996; Stringer, 1998).

1. Epileptogenesis

Seizures may result from primary or acquired disturbances of central nervous system (CNS) function, from metabolic derangements, or from a variety of systemic diseases (Alldredge, 1992; Avoli, 1997).

Identification of the cause of seizures is of primary importance in the determination of subsequent management. If precipitating factors identified are amenable to therapeutic intervention, then specific treatment should be instituted to correct the underlying cause (Alldredge, 1992). In addition, many physiologic disturbances to brain function can produce seizures.

1.1 genetic cause

In the last two decades, increasing attention has been directed to genetics in attempting to determine the underlying pathophysiology of several epilepsies. The hereditary basis of many epilepsy syndromes has long been suspected, but progress in identification of the molecular genetic defects has been slow (Trescher and Lesser, 2000). Febrile seizure is a major genetic component of epilepsy. A family history of febrile seizures consistently emerges as the major risk factor for a first febrile seizure and twin (Berg and Shinna, 1995). Most of the common epilepsy syndrome show complex rather than simple inheritance patterns, which makes linkage analysis difficult (Berkovic and Scheffer, 1998; Trescher and Lesser, 2000).

1.2 Pathologic cause

Seizure can occur in patients with almost any pathologic processes that affect the brain. It occur frequently in acute neurologic disorders (e.g., meningitis) or medical conditions (e.g., hypoxia and nonketotic hyperglycemic coma). These include in

cerebral trauma, infections of CNS, brain tumors, cerebrovascular lesion, congenital malformations and metabolic disorders (Avoli, 1997; Gilroy, 2000).

1.3 Biochemical cause

Epileptic seizures occur as a result of imbalance between inhibitory and excitatory neurotransmitter systems, although the exact mechanisms underlying this imbalance remain uncertain. The highest incidence of epilepsy is in childhood, which implies that the immature brain is more prone to seizure than the mature brain (Meldrum, 1995; Holmes, 1997). Loss of GABA has long topped the list of potential epileptogenic factors. In addition, enhanced glutamatergic excitation is another potential epileptogenic mechanism that has received much attention in recent years, particularly with respect to the role of the *N*-methyl-*D*-aspartate (NMDA) type glutamate receptor which mediated activity appears to contribute synaptic drive associated with epileptiform events (Schwartzkroin, 1997).

2. Classification of Epilepsy

Epilepsy encompasses a heterogeneous group of disorders with multiple cause and manifestations. Classification has been attempted on the basis of clinical events, EEG characteristics, etiology, pathophysiology, anatomy, or age. The International League Against Epilepsy (ILAE) introduced a classification scheme in 1969 and published a revised version in 1981 (Dreifuss, 1997; Trescher and Lesser, 2000).

The ILAE classification recognizes two broad categories of seizures: those that arise in part of one cerebral hemisphere and are accompanied by focal electroencephalographic abnormalities (partial, or focal seizures) and those with clinical and electroencephalograph manifestations essentially simultaneous involvement of all or large parts both cerebral hemispheres from the beginning (generalized onset seizures) (Table 1). It is important to classify the kind of seizure in order to choose the most effective therapy (Brodie and Dichter, 1996).

Table 1. Classification of epilepsy (from Dreifuss, 1997)

I. Partial seizures (seizures beginning locally)

- A. Simple partial seizures (consciousness not impaired)
  1. With motor symptoms
  2. With somatosensory or special sensory symptoms
  3. With autonomic symptoms
  4. With psychic symptoms
- B. Complex partial seizures (consciousness impaired)
  1. Simple partial onset followed by impaired consciousness
     - a. With simple partial feature as in A. 1-4
     - b. Without automatisms
  2. With impairment of consciousness at onset
     - a. With no other features
     - b. With partial feature as in A. 1-4
     - c. With automatisms
- C. Partial seizures evolving to secondarily generalized seizures

II. Generalized seizure

- A. Absence seizure
  1. Absence seizure
  2. Atypical absence
- B. Myoclonic seizures
- C. Clonic seizures
- D. Tonic seizures
- E. Tonic clonic seizures
- F. Atonic seizures

III. Unclassified epileptic seizures

Modified from Commission on Classification of the International League against Epilepsy

3. Amino acid neurotransmitters in epilepsy

The principal conclusion is that augmented activity of the excitatory amino acid glutamate features in most studies, with aspartate, once a front-runner, now trailing behind whether this exaggerated excitatory activity is amplified by a reduction in inhibitory activity in epileptic foci, due for instance, to loss of GABAergic interneurones, or a reduction of γ-aminobutyric acid (GABA) release or its effectiveness at its receptors, remains controversial. The fact that raising brain GABA levels with agents that block its breakdown (e.g. γ-vinyl-GABA, vigabatrin) leads to suppression of seizures is not in itself indicative of a causal (etiological) role of a reduction in tissue levels or reduced rates of endogenous GABA release in epilepsy. Such a reduction would have to be demonstrated to occur in patients with the disease (Bradford, 1995).

3.1 Inhibitory amino acid neurotransmitters

γ-aminobutyric acid (GABA) and glycine are the major inhibitory neurotransmitters in the CNS. Glycine plays a significant role in the spinal cord and brain stem. GABA is predominant in cerebral cortex, hippocampus, thalamus, basal ganglia and cerebellum.

3.1.1 γ-aminobutyric acid (GABA)

GABA is now recognized as the major inhibitory neurotransmitter within the CNS, but actual proof of this awaited in identification of specific receptors for GABA in the postsynaptic membranes adjacent to presynaptic terminals and a demonstration that vesicles within the synaptic terminals of neurons that synthesize GABA (GABAergic neurons) selectively accumulate and concentrate GABA. The release of neurotransmitter molecules from synaptic terminals is quantal, which implies that transmitter molecules are released from synaptic vesicles into the synaptic cleft by a exocytotic process (Shank, Smith-Swintosky and Twyman, 2000). Numerous steps in GABA synaptic function are relevant to epileptogenesis: (a) GABA a synthesis: (b) GABA release: (c) GABA transport: and (d) activation of receptors (Olsen and Avoli, 1997). The action of GABA are

mediated by two different receptor classes that have been defined pharmacologically; $GABA_A$, and $GABA_B$. $GABA_A$ is an ionotropic receptor whereas $GABA_B$ is a metabotropic receptor (Sieghart, 1995).

$GABA_A$ receptor

The $GABA_A$ receptor (Fig 1) is a member of the superfamily of ligand-activated ion channels in the cell membrane. GABA type A ($GABA_A$) receptor are most closely related to strychnine-sensitive glycine receptors, more distantly related to acetylcholine nicotinic receptors and serotonin 5-hydroxytryptamine (5-HT) type receptors, and even more distantly related to glutamate ionotropic receptors (AMPA and kainate receptors and NMDA receptors) (Ortells and Lunt, 1995; Shank, Smith-Swintosky and Twyman, 2000). $GABA_A$ receptors are heteropentameric protein complexes, which when activated undergo a series of conformational changes that form an open channel (pore) selectively permeable to anions, specifically chloride anion (Cl^-) and to a lesser degree (HCO_3^-). Receptor activation normally results in an influx of Cl^- which rapidly and transiently hyperpolarized the membrane, a process generally referred to as the generation of an inhibitory postsynaptic potential. The increase in Cl^- flux also decreases the resistance of the membrane, which acts as a shunt to impede the ability of depolarizing excitatory postsynaptic potentials to elicit action potentials (nerve impulses) (Shank, Smith-Swintosky and Twyman, 2000).

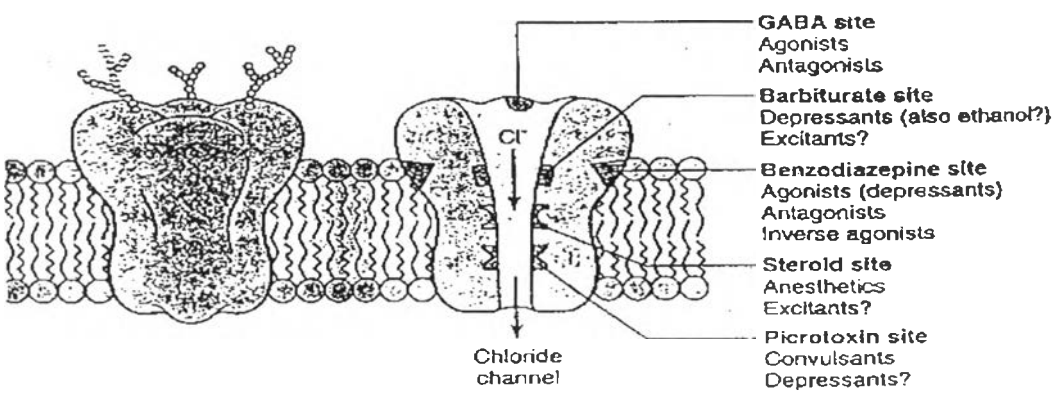

Figure 1. Structure of $GABA_A$ receptor complex

(From Olsen and Delorey, 1999)

$GABA_A$ receptors (Fig 1) are heteromeric in that the receptor can comprise at least five types of subunit proteins, termed α, β, γ, δ, and ρ. It is pentameric in that each receptor has a total of five proteins. The different subunits and the different subtypes of each subunit from which a particular type of receptor is formed can influence the physiological properties of the receptor (e.g., channel open time and rate of desensitization) as well as susceptibility to pharmacological agents (Hevers and Luddens, 1998; Shank, Smith-Swintosky and Twyman, 2000). Many clinically useful drugs are known to bind to benzodiazepine or barbiturate sites. These sites are allosteric to the GABA binding site. An unusual characteristic of the benzodiazepine site is that drugs binding to it can exert either a positive modulatory (an agonist) or negative modulatory (an inverse agonist) (Shank, Smith-Swintosky and Twyman, 2000).

$GABA_B$ receptors

$GABA_B$ receptors are present in most regions of the mammalian brain on presynaptic terminals and postsynaptic neurones. Activation of presynaptic $GABA_B$ receptors located on GABA-containing nerve terminals (autoreceptors) or terminals of various other neurones (heteroreceptors) suppresses the release of neurotransmitter, whereas the stimulation of postsynaptic receptors produces a prolonged neuronal hyperpolarization. The former mechanism appears to be mediated by inhibition of an inward Ca^{2+} conductance whereas the latter is produced by an increase in K^+ conductance. In both cases the $GABA_B$ receptor is coupled via G proteins to its conductance mechanism (Mohler and Fritschy, 1999). $GABA_B$ receptors are G-protein coupled receptor in which its activation initiates guanosine triphosphate (GTP) hydrolysis and thereby causes dissociation of the G-protein subunits (α,β,γ) from the receptor (Shank, Smith-Swintosky and Twyman, 2000).

3.1.2 Glycine

Glycine is present throughout the CNS but is more prevalent in the brainstem and spinal cord, which are the primary areas where it appears to serve a transmitter function. In all tissues, glycine is a major constituent of protein and several peptides such as glutathione. Virtually all criteria required to establish glycine as a

neurotransmitter have been met. These include the identification of specific receptors in the postsynaptic membrane adjacent to presynaptic terminals and a demonstration that vesicles within the synaptic terminals of presumptive glycinergic neurons selectively accumulate and concentrate glycine. Anatomicallly, glycinergic neurons are usually small interneurons, predominantly located in the brainstem and spinal cord, and often functionally associated with α-motorneurons (Shank, Smith-Swintosky and Twyman, 2000).

3.2 Excitatory amino acid neurotransmitters

Historically, the most compelling evidence that glutamate and aspartate function as neurotransmitters came from the observation that at low concentrations they excite virtually every neuron in the CNS. In the adult CNS, L-glutamate and L-aspartate are the most likely candidates for neurotransmitter action at excitatory amino acid receptors, and these amino acids are used by some of the most widely distributed neuronal types. Glutamate and aspartate are present in high concentrations in the CNS and are released in a Ca^{2+}- dependent manner upon electrical stimulation in vitro. (Dingledine and McBrain, 1999). Glutamate receptor subtypes are distinguishable by biochemical, electrophysiological, and pharmacological criteria. Multiple receptor gene families mediate the versatile and widespread function of glutamate signaling. Based on their mode of function, glutamate receptors (Fig 2) have been divided into two major groups; metabotropic receptors which are couple to second messenger pathway through G-proteins and ionotropic receptors which are ligand-gated ion channels (Chapman, 1998; Dodd et al., 2000).

Ionotropic glutamate receptor (GluR)

The ionotropic glutamate receptor (GluR) family is composed of closely related subunits that combine to form receptors that are selectively activated by agonists. These receptors allow the flow of ions across into three major groups according to their respective preferential activator namely *N*-methyl-*D*-aspartate (NMDA). α-amino acid-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainic acid (KA) (Homes, 1997; Chapman, 1998). The KA and AMPA receptors share many characteristics and are

collectively refered to as the non-NMDA receptors. Glutamate binds to all these receptors. The NMDA receptor binds NMDA, aspartate and glutamate; the AMPA receptor binds AMPA, KA or glutamate; the KA receptor binds KA or glutamate. Binding of an agonist to any of these glutamate receptor subtypes leads to a conformation change in the ionic channel linked to the receptor with subsequent flow of cations into the neuron. Channels of the NMDA receptor allow influx of Na^+ and Ca^{2+} ions while AMPA and kainate receptors admit Na^+ (and to a lesser extent Ca^{2+}) ions (Chapman, 1998; Dingledine and McBrain, 1999; Dodd et al., 2000)

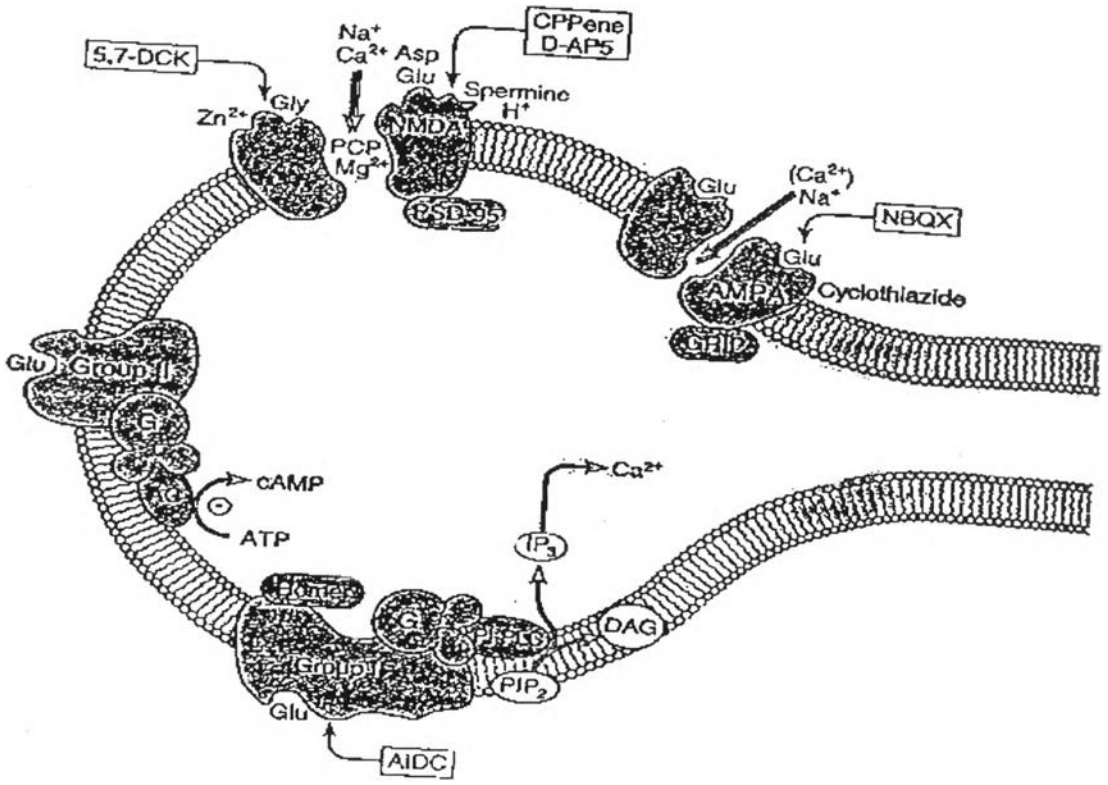

Figure 2. Schematic views of four types of glutamate receptor

(From Dingledine and McBrain, 2000)

The NMDA receptor channel has slower kinetics than AMPA/KA receptors and mediates Na^+ and Ca^{2+} influx. The slow kinetics of channel opening allows both summation of glutamate response and a large influx of calcium into cell. Increase in intracellular calcium concentration is believed to be critical for many of the proposed roles of the NMDA receptor. Ion flux through the NMDA receptor is voltage dependent. When the

cell is at resting potential, Mg^{2+} binds within the ion channel and block the cation flux. It is likely that synaptically release glutamate first activates AMPA/KA receptors, thereby causing depolarization of the post-synaptic cell and release of Mg^{2+} ion such that other cations can move through the NMDA receptor ion channel (Lipton and Rosenberg, 1994; Chapman, 1998; Dodd et al., 2000).

Metabotropic glutamate receptor (mGluR)

To date, there are eight metabotropic glutamate receptors (mGlu1-8) with known molecular sequence and can be studied in expression system. Many of the effects mediated by diacylglycerol or cAMP are related to altered phosphorylation of various enzymes receptors or transporters that give rise to prolonged function changes. Activation of Group I (mGlu1 and mGlu5) receptors can potentiate NMDA and AMPA responses. Glutamate release can be enhanced by mGlu1 activation. Group II (mGlu2 and mGlu3) and Group III (mGlu4, mGlu6, mGlu7 and mGlu8) receptors act presynaptically to decrease glutamate release (Chapman, 1998; Dodd et al., 2000).

4. Therapy of epilepsy

4.1 The surgical treatment of epilepsy

Approximately 20-30% of patients with epilepsy do not respond to anticonvulsant drugs. When medication is unsuccessful in controlling seizures, properly selected patients have an excellents chance of achieving improved or complete seizure control with surgery (Trescher and Lesser, 2000).

The essential factor in modern epilepsy surgery is the input of a multidisciplinary team of highly trained individuals working together in an epilepsy center. A detailed presurgical evaluation is mandatory to identify the offending area of the brain responsible for the seizure activity and to ensure a high rate to success while avoiding new neurological on cognitive deficits (Gilroy, 2000).

4.2 Drugs for treatment of epilepsy

In the absence of a specific etiological understanding in any of the epilepsies or epileptic syndromes, approaches to drug therapy of epilepy must necessarily be directed at the control of symptoms, i.e. the suppression of seizures (Brodie and Dichter, 1996; Loscher, 1998). The goal of antiepileptic drug therapy is to prevent seizures while minimizing side effects, using the simplest drug regimen. If seizures continue after the start of therapy and further, increases in dose are inadvisable because of dose-related side effects. One should try at least one and sometimes another alternative drug as monotherapy before considering the use of two drugs simultaneously. Discontinuation of antiepileptic medication after several seizure-free years depends on the diagnosis (type of seizure and epileptic syndrome), cause and response to therapy. Antiepileptic drugs may be discontinued in patients with certain epileptic syndromes but should be continued for life in patients with others, such as recurrent seizures secondary to a structural lesion (Stringer, 1998).

Development of new antiepileptic drug

There are at least three preclinical strategies which are used for development of new anticonvulsant drugs; (1) random screening of newly synthesised 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 (Upton, 1994; Loscher, 1998).

Several new antiepileptic medications have been recently developed on the basis of what is known about cellular and molecular mechanism controlling neuronal and epileptic synchronization (Avoli, 1997). 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, 1996; Loscher, 1998). This knowledge generated several rational strategies for drug development, aimed to identify new anticonvulsant drugs with high specificity/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 (Upton, 1994; Loscher, 1998). 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; White, 1997; Loscher, 1998).

Gabapentin (GBP)

GBP was originally synthesized as a GABA analogue, capable of readily traversing the blood-brain barrier. However, direct effects have not been demonstrated. GBP nevertheless appears to increase GABA turnover in various regions of the rat brain. A wide range of actions on enzymes involved in the synthesis and metabolism of glutamate and GABA have recently been described, including inhibition of branched-chain amino acid aminotransferase (which converts branched-chain amino acid and α-ketoglutarate to α-ketoacids and glutamate) at relatively low concentrations. A weak inhibitory action agoinst GABA transminase is seen at high concentrations of GBP. Like valproate, GBP has a broad spectrum of anticonvulsant activity (Meldrum, 1996; Rho and Sankar, 1999).

Vigabatrin (VGB)

VGB (γ-vinyl-GABA) is perhaps the first antiepileptic drug developed through rational design rather than through empiric screening in animal seizure models or from serendipitous findings. VGB is an irreversible inhibitor of a pyridoxal dependent enzyme primarily responsible for GABA catabolism, γ-aminobutyric acid transminase (or GABA-T). GABA-T is present in both neurons and glia; however, VGB appears to have a greater affinity for neuronal GABA-T. Inhibition of GABA-T leads to prolonged, elevated levels of GABA is rodent and human brain. Despite these observation, the precise manner in which VGB prevents seizures remains unclear. The antiepileptic drug effect of VGB may relate to the fact that GABA-T inhibition results in a greater releasable pool of GABA, identified in synaptosomes, more than simply to an elevation in whole brain GABA levels. Thus, larger amounts of GABA may be made available and released in the presynaptic

space during epileptic seizures to provide sufficient postsynaptic inhibition of GABA receptors (Meldrum, 1996; White, 1997; Rho and Sankar, 1999).

Felbamate (FBM)

FBM is a novel antiepileptic drug with a broad spectrum of activity in animal seizure models and is effective against many seizure type in humans. FBM reduces sustained repetitive firing in mouse spinal cord neurons in a concentration-dependent manner. This suggests an interaction with voltage-dependent Na^+ channels directly inhibits Na^+ currents in a dose and voltage-dependent fashion has not been determined. Several lines of evidence indicate that FBM acts in part by modulating glutamate receptor function through an action on glycine, a coagonist of glutamate necessary for activation of the NMDA receptor (White, 1997; Rho and Sankar, 1999).

Tiagabine (TGB)

TGB exhibits efficacy against a wide range of animal seizure models and against partial seizures in humans. TGB is another product of rational antiepileptic drug development specifically targeting the GABAergic system. Rather than inhibiting GABA degradation, as is the case with VGB, an alternative approach had been to develop compounds that block GABA reuptake, Thereby increasing synaptic levels of GABA and a consequent enhancement and prolongation of GABA-mediated inhibitory neurotransmission, which is assumed to be the basis of TGB's anticonvulsant activity (Meldrum, 1996; White, 1997; Rho and Sankar, 1999).

Topiramate (TPM)

TPM has multiple actions that contribute to a broad anticonvulsant profile. Effects on voltage-sensitive Na^+ channels and on non-NMDA glutamate receptors may account for efficacy against partial seizures secondarily generalized and for the capacity to prevent seizure spread. The enhancement of GABA-mediated inhibition may contribute to the capacity to raise the seizure threshold and possible effectiveness in absence seizures (Meldrum, 1996; White, 1997; Rho and Sankar, 1999).

Lamotrigine (LTG)

LTG is initially developed as a folate antagonist after the observation the patients with epilepsy treated with antiepileptic drug. LTG has been shown to act by prolonged inactivation of the voltage sensitive Na^+ channel and blockage of predominantly on N and P type calcium channels (Meldrum, 1996; White, 1997; Rho and Sankar, 1999).

Valproic acid

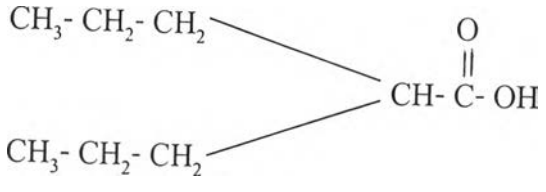

Figure 3. Structural formula of valproic acid

A valproic acid (VPA, Fig 3) is the trivial name for α-propylpentanoic acid (also called n-dipropylacetic acid). Its simple structure of a short-branched fatty acid differs to a great extent from the substituted heterocyclic ring structures characterizing the traditionally used antiepileptic drugs (Loscher, 1999; Johannessen, 2000). VPA is effective in patients with all types of seizures, and especially in those with idiopathic generalized epilepsy. In spite of its wide use for many years, the mechanism of VPA action is still not fully understood (Brodie and Dichter, 1996; Johannesson, 2000). The antiepileptic action of VPA is probably due to a combination of several effects in the CNS because of its wide spectrum of activity against different types of seizures and status epilepticus. The theories proposed that its anticonvulsant effects are brought about by (1) an increase in GABAergic synaptic inhibition through a presynaptic or postsynaptic action (2) a reduction in excitatory synaptic transmission responsibly for synchronization of cell firing which leads to epileptic busting or (3) by reducing repetitive firing of neurons through a direct effect on voltage-sensitive ion channels (Loscher, 1999; Rho and Sankar, 1999; Johannesson, 2000).

Increase in GABAergic synaptic inhibition

The precursor of GABA, L-glutamic acid, is formed from glucose via the glycolytic pathway and Krebs cycle. GABA is synthesized in GABAergic nerve terminal by glutamic acid decarboxylase (GAD) converted glutamate to GABA. GABA is degraded by GABA transminase (GABA-T) to succinic semialdehyde (SSAD) which can either be oxidized to succinic acid by the enzyme succinic semialdehyde dehydrogenase (SSADH) or it can be reduced to gamma-hydroxybutyrate (GHB) (Cooper, Bloom and Roth, 1996; Loscher, 1999).

The GABA-elevating effect of valproate was originally attributed to inhibition of GABA-T. Most studies on inhibition of GABA-T by valproate found that inhibitory effects occurred only at very high concentrations (Loscher, 1999; Rho and Sankar, 1999; Johannesson, 2000). VPA treatment induced a significant inhibition of synaptosomal GABA-T activities in several brain regions, including substantia nigra, hippocampus, hypothalamus, pons, and cerebellum. In addition VPA exerts a more potent inhibitory effect against SSADH, the enzyme responsible for degradation of SSA to succinic acid, than against GABA-T. It has been postulated that accumulation of SSA by inhibition of SSADH either initiates the reverse reaction of GABA-T, thus increasing the levels of GABA via conversion of SSA, or that the increased levels of SSA inhibit the degradation of GABA, since SSA has a strong inhibitory effect on the forward reaction of GABA-T (Loscher, 1999; Johannesson, 2000).

Reduction of excitatory neurotransmission

Some studies have suggested that VPA suppresses glutamate responses and, much more potently, NMDA-evoked transient depolarization in rat neocortex. Attenuation of NMDA receptor mediated excitation is an essential mode of action for the anticonvulsant effect of VPA. In all studies, VPA blocked these responses, indicating that antagonism of NMDA receptor mediated neuronal excitation may be an important mechanism of VPA (Loscher, 1999; Johannesson, 2000).

Effects on sodium channels

The predominant mechanism of action on ion channels, like the other traditionally used anticonvulsive agents (phenytoin and carbamazepine). VPA appears to act at the voltage-dependent Na^+ channel, inhibiting high frequency firing of neurons (Johannessen, 2000). This ability to limit repetitive high frequency firing may certainly contribute to the efficacy of VPA in epilepsy, where excessive high-frequency bursting of neuronal aggregates is seen. Chronic treatment with VPA has been postulated to up-regulate cell surface expression of sodium channels. In cultured hippocampal neurons. VPA (1 mM) reduced peak sodium conductance in a voltage dependent manner, and VPA also retarded the recovery from inactivation. This last finding was not confirmed by Albus and Williamson (1998) who did not find any changes in the refractory period and recovery from inactivation of sodium channels in hippocampal slices with VPA (1 mM). They conclude that at least in the hippocampal slice, the principal antiepileptic action of VPA cannot be explained by its action on voltage dependent sodium channels (Johennesson, 2000).

Valpromide (VPD) is the primary amide of VPA. Studies in mice and rats showed that VPD is a non-teratogenic entity which is more potent that VPA. However, the advantages of VPD over VPA in rodents have no clinical implications, as in human VPD server as a prodrug to VPA. Therefore, there is a need to develop stable VPD analogues and valproyl hydroxamic acid (VHA) is one among several novel derivatives of VPD that were synthesized (Levi, Yagen and Bialer, 1997).

Valproyl hydroxamic acid (VHA)

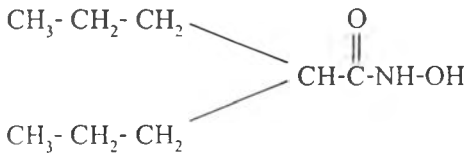

Figure 4. Structural formula of valproyl hydroxamic acid

In previous studies by Levi, Yagen and Bialer (1997), VHA (Fig 4) demonstrated better anticonvulsant activity than VPA, in maximal electroshock (MES) test, because of their greater intrinsic activity but not its better pharmacokinetic characteristics. Pharmacokinetic analysis showed that VHA had the high clearance value. Its mean clearance was 4 and 8 times larger than that of VPA and VPD respectively, the volume of distribution of VHA was similar to that of VPA and VPD. Consequently, VHA had the shortest half-life. However, unlike VPD, VHA was not biotransformed *in vivo* to VPA.

In Thailand, VHA was synthesized by Assistant Professor Dr. Chamnan Patarapanich et al. in 1996 (Thongsathean, 1999). The structure of VHA consist of two parts, one is the branched chain and the other is the structural changes of the substituents attached to the nitrogen of the amide moiety by a hydroxyl. The chemical structure of VHA was designed in expectation for a higher potency and less toxicity than VPA.

The evaluation of anticonvulsant activity was performed in mice using the MES and chemically induced seizure tests. In MES test, VHA was found to be intraperitoneally effective showing the maximal protection around 15 min after pretreatment while the corresponding value for VPA was 30 min. Intraperitoneal administration of VHA demonstrated a higher protection than VPA in MES and bicuculline but was effective as VPA in PTZ tests. The median effective dose (ED_{50}) of VHA were 117, 97 and 153 mg/kg B.W. in MES, PTZ and bicuculline tests respectively, while corresponding values for VPA were 211, 99 and 382 mg/kg B.W. VHA weakly blocked the effect of strychnine exhibiting ED_{50} of 441 mg/kg B.W., while VPA was ineffective.

With regards to toxicity in terms of lethality, the median lethal dose (LD_{50}) of VHA (840 mg/kg B.W.) is slightly higher that of VPA. The median neurotoxicity dose (TD_{50}) as measured by rotorod test were 189 and 260 mg/kg B.W. for VHA and VPA respectively. Furthermore, ED_{50} of VHA had no significant depressant effect on locomotor activity prolongation of barbiturate sleeping time was exhibited by VHA in the dose of 120 mg/kg B.W.

The observation that VHA was not degraded to VPA by brain or liver homogenate as well as that VHA was able to protect the animal against MES test giving the ED_{50} of 102 μM when being administered intracerebroventricularly indicate that VHA is an active anticonvulsant molecule (Thongsathean, 1999). However no attempt has been made by previous investigators to further classify the possible mechanism of VHA. Therefore, to give insight into the effect of VHA on brain amino acid which may underlie its anticonvulsant activity. The present studies aim to investigate the effect of VHA on cortical excitatory and inhibitory amino acid neurotransmitter in freely moving rats.