Chapter 1
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 is a common disorders of brain affecting at least 20 to 50 million people worldwide. The epilepsies are characterized by spontaneous disturbances in the normal electrical activity of the brain associated with changes in behavior (l.öscher , 1993a; Dichter, 1997).

1. Epileptogenesis

Epilepsy has no single cause but is rather a multifactorial condition reflecting pathologic cause, including acquired injuries and genetic abnormalities. In addition, many physiologic disturbances of brain function can produce seizures (Avoli, 1997).

1.1 Pathologic cause

Seizures are a common symptom of disturbed brain function and thus 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 central nervous system (CNS), brain tumors, cerebrovascular lesions, congenital malformations and metabolic disorders (Scheuer and Pedley, 1990; Avoli, 1997).

1.2 Genetic cause

It has long been known that epilepsy is a major genetic component to febrile seizure. A family history of febrile seizures consistently emerges as the major risk factor for

a first febrile seizure and twin (Berg and Shinna, 1995). Although autosomal dominant and recessive models of heritance have been proposed, most evidence favors a complex mode of inheritance, that is polygenic or multiplefactorial (Buchhalter, 1993; Berkovic and Scheffer, 1998).

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; Scott and Neville, 1998). 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

Classification of epilepsy is complicated and can be based on etiology, pathology, and age of onset, clinical seizure, electroencephalogram (EEG) findings, or prognosis.
A revised classification of individual seizure types was accepted in 1981 by the General Assembly of the International League Against Epilepsy (ILAE) (Porter, 1993; Dreifuss, 1997, Table 1). 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). It is Important to classify the kind of seizure in order to choose the most effective therapy (Scheuer and Pedley , 1990 ; 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 conscious
    - 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 basic mechanism of neuronal excitability is the action potential, a hyperexcitable state can result from an increase in excitatory synaptic neurotransmission and a decrease of inhibitory neurotransmission (Schwartzkroin, 1997; Ure and Perassolo, 2000).

.

3.1 Inhibitory amino acid neurotransmitters

Glycine and γ-aminobutyric acid (GABA) 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)

γ-aminobutyric acid (GABA) is known to be a major inhibitory neurotransmitter in the brain. It exerts an inhibitory action in all of brain structures and plays a crucial role in preventing the spread of excitatory activity (Kuriyama, Hirouchi and Nakayasu, 1993; Holmes, 1997; Barnard et al., 1998). The actions of GABA are mediated by two different receptor classes that have been defined pharmacologically: $GABA_A$, and $GABA_B$. $GABA_A$ is a ionotropic recepter whereas $GABA_B$ is a metabotropic receptor (Sieghart, 1995; Barnard et al., 1998) .

$GABA_A$ receptor

$GABA_A$ receptor (Figure 1) is stimulated by muscimol and isoguvacine and inhibited by bicucullin in a competitive manner (Sieghart, 1995; Hevers and Lüddens, 1998). At the postsynaptic level, the activation of $GABA_A$ receptor induces the fast inhibitory postsynaptic potential (IPSP) which is directly associated with a Cl^- ion channels (Sieghart, 1995; Olsen and Avoli, 1997). The molecular weight of the $GABA_A$ receptor complex was

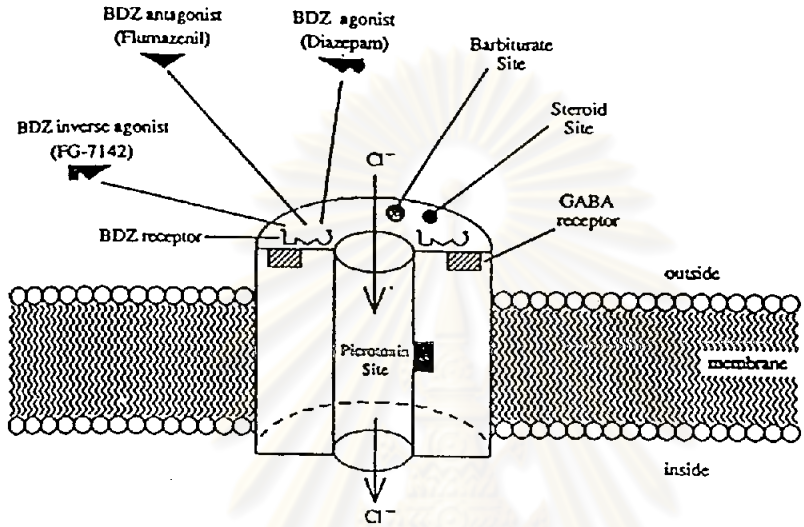

Figure 1 Structure of GABA $_A$ receptor complex

(from Cooper, Bloom and Roth, 1996)

estimated to be approximately 300 kDa. The $GABA_A$ receptor is a member of a superfamily of ligand-gated ion channels that include the nicotinic acetylcholine receptor (nAchR), the glycine receptor, and the 5-HT_3 receptor (Ortells and Lunt, 1995). The $GABA_A$ receptor is a macromolecular protein containing binding sites for at least GABA, picrotoxin, neurosteroids, barbiturates and benzodiazepines (Barnard et al., 1998; Hevers and Lüddens, 1998). Barbiturate derivatives, which are classified as one of the sedative-hypnotic drugs, are known to enhance the action of GABA at the $GABA_A$ receptor by increasing the average channel open duration but not alter receptor conductance or

opening frequency. On the other hand, benzodiazepines, anxiolytic, sedative-hypnotic and muscle relaxant drugs enhance the action of GABA at the $GABA_A$ receptor by increasing the opening frequency of Cl^- channels (Sieghart, 1995; Hevers and Lüddens, 1998)

Recently, molecular cloning revealed an ever increasing number of subunit isoforms, that have been classified according to their degree of amino acid identify as α, β, γ, δ, ρ, and most recently ε. For mammals, these are α1-6, β1-3, γ1-3, δ, ε, and ρ 1-3 (Kuriyama, Hirouchi and Nakayasu, 1993; Hevers and Lüddens, 1998).

$GABA_B$ receptor

$GABA_B$ receptor, the purified $GABA_B$ receptor protein is about 80 kDa in its molecular weight. In the mammalian CNS two electrophysiological detectable effects of $GABA_B$ receptor activation are well established (1) activation of potassium currents and (2) modulation of calcium currents. The cloning of isoforms of the $GABA_B$ receptor revealed sequence similarity to the metabotropic glutamate receptors. More recently, functional receptors are heterodimers of $GABA_B$-R_1 and R_2 subunit (Kaupmann et al., 1997; White et al., 1998a). The $GABA_B$ receptors, which are insensitive to bicucullin and sensitive to baclofen were revealed, and it was found that this receptor is one of the metabotropic types of receptors, which indirectly coupled to K^+ or Ca^{2-} channel via GTP – binding protein. The activation of the $GABA_B$ receptor is known to induce slow IPSP (Kuriyama, Hirouchi and Nakayasu, 1993; Barnard et al., 1998). The $GABA_B$ receptors indeed are localized to nerve endings where they inhibit transmitter release. When they are located at excitatory terminal, which their activation inhibits glutamate release thus, the overall effect is a decrease in excitation. However, when $GABA_B$ receptors are located at inhibitory terminals (so-called autoreceptors), their activation will cause a decreased release of GABA, which may result in an excitatory influence (Olsen and Avoli, 1997).

3.1.2 Glycine

Glycine is a short chain amino acid, which shows powerful inhibitory action. It is primary restricted to the spinal cord and brain stem. Glycine is one of the most ccmmon amino acid in the CNS and is metabolically derived from serine by the action of serine-transhydroxylmethylase. The primary source of serine is glucose via the enzymes 3-phospho-D-glycerate and 3-phospho-serine. Glycinnergic inhibition mediates most of the fast inhibitory synaptic transmission. Spinal cord inhibitory postsynapstic potential can be mimicked by the application of glycine and glycine receptors are permeable to chloride ion. The NMDA receptor is unique in its requirement for the co-agonists of glutamate and glycine for complete activation of the channel (Cooper, Bloom and Roth, 1996)

3.2 Excitatory amino acid neurotransmitters

Glutamate is the primary neurotransmitter in the mammalian CNS and has been implicated as a potent neurotoxin. In brain, L-glutamate is synthesized in the nerve terminals from two sources (Figure 2): from glucose via the Krebs cycle and transamination of α-oxoglutarate and from glutamine that is synthesized in glia cell, transported into nerve terminal and locally converted by glutaminase into glutamate. In the glutamate-containing nerve terminal, glutamate is stored in synaptic vesicle, and on depolarization of the nerve terminal it is released by a calcium-dependent exocytotic process (Cooper, Bloom and Roth, 1996; Dichter and Wilcox, 1997). 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 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 the neuronal membrane. The ionotropic glutamate receptors can be further divided 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) (Holmes, 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 or 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; Dodd et al, 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).

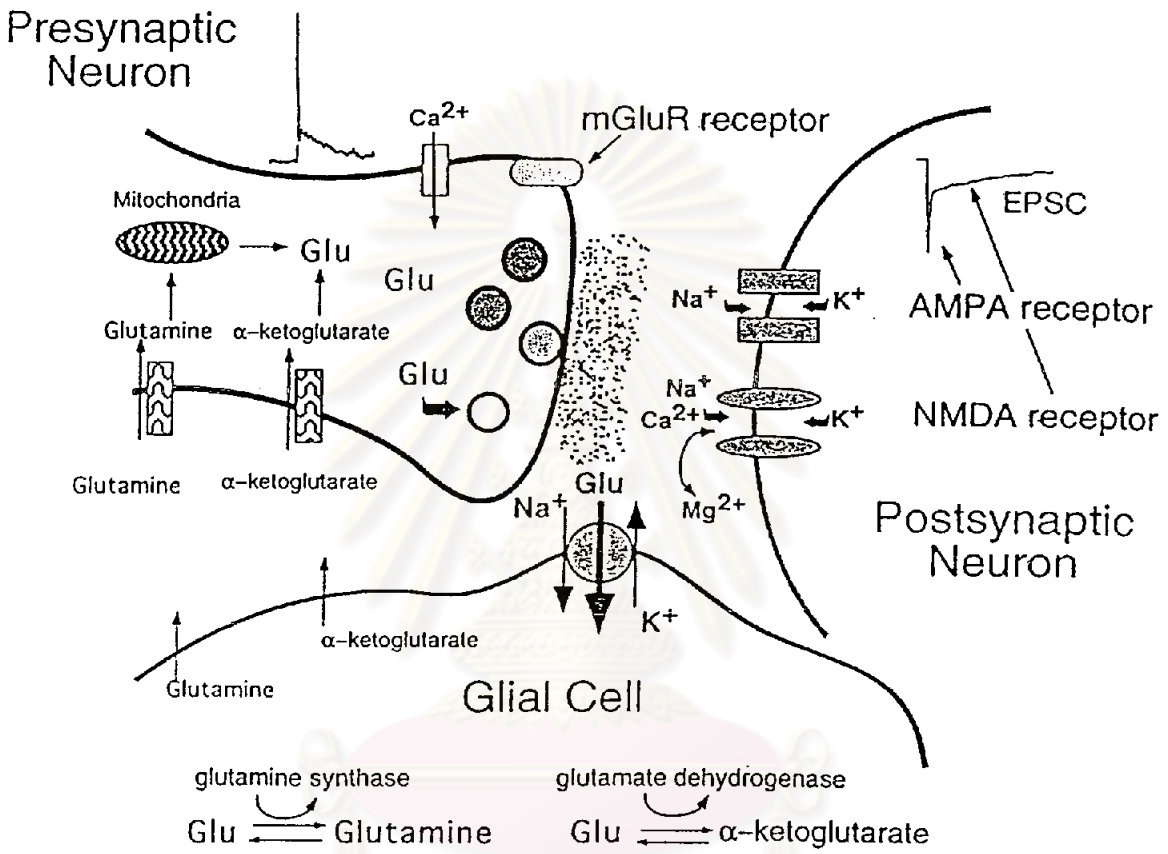

Figure 2 Schematic diagram of Glutamate

(From Dichter and Wilcox, 1997)

Metabotropic 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 (mGlu 1 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

Epilepsy surgery is an effective therapy for many patients with refractory partial seizure fail to response to antiepileptic drugs. Since the beginning of epilepsy surgery the outcome of surgical treatment of epilepsy has improved steadily. The morbidity is less and there is more seizure - free outcomes in most modern series of surgery. Today surgical patients are benefited by improvement in general surgical care (Boling and Oliver, 1998).

Three criteria can be used to identify the ideal candidate for surgery, (1) the seizures begin in an identifiable and restricted cortical area, (2) the surgical excision can encompass the bulk of the epileptogenic region, and (3) the necessary resection will not impair neurologic function (Scheuer and Pedley, 1990).

4.2 Drugs for treatment of epilepsy

Antiepileptic drugs can control either the initiation or maintenance of the epileptic discharge or spread within the brain. However, there remains a therapeutic approach that should be further exploited. In the past, empirical drug screening and at

times serendipity represented the main resource for identifying molecules with antiepileptic properties. Although there are a wide variety of specific molecular targets, all anticonvulsant drugs ultimately must exert their actions by altering the activity of the basic mediators of neuronal excitability: voltage – and neurotransmitter – gated ion channels (Rogawski and Porter, 1990; MacNamara, 1996). Anticonvulsant drugs can be divided mechanistically into at least three classes based on ability (1) to block sustained high - frequency repetitive firing of action potentials by blockage of voltage– dependent Na^+ channels, (2) to enhance GABAergic inhibition and (3) to block slow, pacemaker – driven, repetitive firing by blocking T Ca^+ currents (Macdonald and Kelly, 1993).

Development of new antiepileptic drug

There are at least three preclinical strategies which are used for development the new anticonvulsant drugs: (1) random screening of newly synthesized chemical compounds of diverse structure 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 pathological events involved in seizures or epilepsy (Upton, 1994).

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. The past decades have witnessed an increase in our knowledge on the pathophysiology of brain disease and the basic mechanisms of drug activity (Dichter, 1994; Avoli, 1997). This knowledge generated several rational strategies for drug development, aimed to identify new anticonvulsant drugs. They are (1) enhancement of GABA – mediated neuronal inhibition by acting on GABA transport, metabolism, or receptor activity, (2) reduce of glutamate - mediated neuronal excitation and other excitatory amino acid and (3) modulation of Na^+, K^+ and particularly Ca^{2+} ion channels. 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).

Vigabatrin (VGB) is a GABA analog (VGB, γ- vinyl - GABA) which inhibits GABA catabolic enzyme GABA–transaminase (GABA-T). VGB binds to GABA-T and permanently inactivates the enzyme, thereby increasing brain GABA levels and enhancing GABAergic neurotransmission. The consequent increased activity of GABA on postsynaptic GABA receptors results in increased inhibition of neurons involved in seizure activity and represents the most likely basis of VGB's clinical antiepileptic activity (Meldrum, 1996; White, 1997; Rho and Sankar, 1999).

Tiagabine (TGB) exhibits efficacy against a wide range of animal seizure models and against partial seizures in human. TGB acts on GABA neurotransmission by inhibiting GABA uptake thus prolong the synaptic inhibitory effect of GABA (Meldrum, 1997; White, 1997; Rho and Sankar, 1999).

Gabapentin (GBP) is a specific GABA analog originally designed to mimic the steric conformation of GABA and to have high lipid solubility in order to penetrate the blood-brain barrier. However, direct effects have not been demonstrated. GBP has been shown to increase GABA turnover in several regions of 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 acids and α–ketoglutarate to α-keto acids and glutamate) at relatively low concentrations. Like valproate, GBP has a broad spectrum of anticonvulsant activity (Meldrum, 1996; Rho and Sankar, 1999).

Felbamate (FBM) is a novel antiepileptic drug with a broad spectrum of activity in animal seizure models and is effective against many seizure types in human. FBM reduces sustained repetitive firing in culture mouse neurons and may have an act on the voltage – dependent Na^+ channel. FBM is the first pharmacological agent reported both to act on GABA mediated inhibition and act in part by moderating glutamate receptor function though an action on glycine binding site, a co-agonist of glutamate necessary for activation of the NMDA receptor (White, 1997; Rho and Sankar, 1999).

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

Lamotrigine (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 prolonging 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

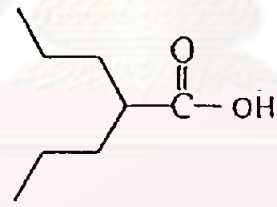

Figure 3 Structural formula of Valproic acid ,VPA

A valproic acid (VPA, Figure 3) is a simple structure of a short branched chain fatty acid and differs structurally from other antiepileptic drugs. VPA is the most commonly used antiepileptic drug in the treatment of generalized and partial seizures in adults and children (Davis, Peter and McTavish; 1994; Löscher, 1998). Despite of its wide spectrum of anticonvulsant activity against different seizure types, the mechanism of VPA action is still not fully understood (Löscher, 1998; Johannessen, 2000). 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 responsible for synchronization of cell firing which leads to epileptic bursting or (3) by reducing repetitive firing of neurons through a direct effect on voltage-sensitive ion channels (Löscher, 1993b; Davis, Peter and McTavish, 1994; Johannessen, 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 glutamate decarboxylase (GAD) converted glutamate to GABA then GABA is degraded by GABA transaminase (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)).

VPA significantly enhance the activity of GAD in the medulla, pons, cerebellum and midbrain regions of rats, and partially relived the suppression of GAD activity caused by chronic GABA-T inhibition in whole mouse brain (Phillips and Fowler, 1982). In addition, it has been postulated that accumulation of SSAD by inhibition of SSADH either initiates the reverse reaction of GABA-T, thus the increase level of SSAD inhibit the degradation of GABA, since SSAD has a strong inhibitory effect on the forward reaction of GABA-T (Godin et al., 1969; Van Der Laan, De Boer and Bruinvels, 1979, Nau and Löscher, 1982)

Reduction of excitatory neurotransmission

Some studies have suggested that VPA causes decrease of excitatory transmission in brain: VPA acts by reducing the number of action potentials elicited by application of NMDA in neocortical neurons in the rat (Zeise et al., 1991). The release of aspartate, being localized in glutamatergic nerve terminal, has been found to be inhibited by VPA in the dose of 200-400 mg/kg (Crowder and Bradford, 1987).

Effects on sodium channels

The predominant mechanism of action on ion channels, is similar to the other traditionally used anticonvulsive agents (phenytoin and carbarmazepine). VPA diminishes sustained repetitive firing of culture mouse spinal cord and neocortical neurons, implying actions on voltage-gated sodium channels (Mclean and Macdonald, 1986). The most likely explanation for this effect of valproate would be a use-dependent reduction of inward sodium current. However, Albus and Williamson (1998) did not find any changes in the refractory period and the recovery from inactivation of sodium channels in hippocampal slices with VPA (1mM). They concluded that at least in the hippocampal slice, the principle antiepileptic action of VPA could not be explained by its action on voltage dependent sodium.

The incident of toxicity associated with the clinical use of VPA is low but two rare toxic effects, idiosyncratic fatal hepatotoxicity and teratogenicity, necessitate precaution in risk patient population (David, Peters and McTavish, 1994).

Valproyl morpholine (VPM)

Valproyl morpholine (VPM) is a valproate derivative, which was synthesized by Lauluk Lomlim and Badin Tiwsuwan (1997) in a search for a valproate analog.(Figure 4).

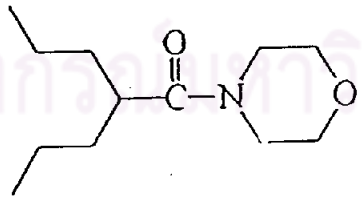

Figure 4 Structural formula of valproyl morpholine, VPM

As previously reported in primary screening for anticonvulsant activity of VPM (Badin Tiwsuwan,1997), it was found that VPM was effective only in the MES but not in the PTZ models. Comparatively, VPM was more potent than VPA in the MES test. Furthermore, it was likely that VPM might possess pronounced depressant effect on the CNS as predicted by its effect on potentiation of barbiturate sleeping time.

MES test is the most wildly used screening test for anticonvulsant activity because of its simplicity, reproducibility and predictive value of clinical efficacy. Efficacy in the MES model correlates with efficacy in suppressing tonic-clonic generalized seizures. All the currently available drugs that are clinically effective in the treatment of generalized tonic-clonic seizures are effective in the MES test. In contrast, PTZ test examines the ability of a compound to raise seizure threshold and is predictive of drug efficacy in absence seizure(Rogawski and Porter, 1990; White, 1997; Löscher, 1998). Furthermore, it has been suggested that these two tests may actually evaluate the effect of drugs on two separate anatomical structures (Browning, 1992). Both of the tests are recommended as primary screening tests in Antiepileptic Drug Development (ADD) program which was established in the U.S.A. in 1974 by collaborations between government, pharmaceutical industry and academia (White et al., 1998b). Thus, this study aims to evaluate anticonvulsant activity, expressed as the median effective dose(ED_{50}), of VPM in both the MES and PTZ models in parallel with its toxicity in a battery of toxicity testing as suggested in the ADD program (Cereghino and Kupferberg, 1993). Furthermore a possible effect of VPM on the levels of cortical excitatory and inhibitory amino acid neurotransmitters of freely moving rats would also be investigated.