Chapter I

Introduction

Epilepsy

Epilepsy is not a disease but a syndrome of many different cerebral disorders characterized by recurrent seizure. An abnormal discharge of a set of neuron demonstrating excessive fluctuations in cerebral electrochemical balance accounts for the clinical manifestation of epilepsy (Hauser, Annegers, and Anderson, 1983; Fukuzako and Izumi, 1991).

Two primary features characterize epileptiform activity, either one of which may be sufficient to produce seizure activity. The first is hyperexcitability of multiple neurons within a population. Almost all of the means by which cells can become hyperexcitable involve normal basic mechanisms that have been deregulated. The second is hypersynchrony. Cell populations may be synchronized, even in the absence of hyperexitability and still yield seizure activity (Schwartzkroin, 1993).

Epilepsy is one of the most common neurological disorders; if we estimate that 1% of the world population has epilepsy, then about 50 million persons worldwide suffer from this disorder. Globally, the prevalence of epilepsy was nearly 0.5-1.0% of every age (Porter, 1993).

1. Epileptogenesis

One of the major outcomes of research over the past 20 years is the"discovery" that no single mechanism underlies epileptiform activity. Indeed each form of epilepsy is probably generated by different mechanisms.

1.1 Pathologic cause

Seizure can occur in patients with almost any pathologic processes that affect the brain. It can be resulted from congenital malformations, infections, tumors, vascular diseases, degenerative disease, injury or heredity. In more than three-quarters of patients with epilepsy, the seizures begin before the age of 18 years. The reason for this age of onset is not clear (Porter, 1993).

1.2 Genetic cause

Epilepsy has been considered a genetic disease for centuries. The chromosomal location of genes determining several human syndromes of epilepsy have been approximately determined. "Epilepsy genes" that might directly enhance excitatory neurotransmission include those coding for: (1) enzymes involved in glutamate and aspartate metabolism; (2) glutamate and other amino acid transporter; and (3) glutamate receptor. The overexpression of excitatory receptor can be a direct cause of epilepsy that has recently been demonstrated in rats by the intrahippocampal injection of a herpes virus vector carrying a GluR6 subunit of the kainate receptor. This leads in 3 to 4 hours to the appearance of limbic seizures that recur over several weeks (Meldrum, 1994).

The genetic susceptibility to seizure is normally distributed in general population and that there is a threshold above with the condition becomes clinically evident. Recurrent seizure are thought to be resulted from a genetic predisposition which appears to be the most significant in patients with various primary epilepsies (Menkes, 1990; Hopkin, 1993).

1.3 Biochemical causes

For the majority of human epileptic syndromes, the molecular and cellular basis for the epileptic activity remain largely conjectural. The principal hypothesis currently concern are defect in γ-aminobutyric acid (GABA) mediated inhibitory process, enhanced or abnormal excitatory synaptic action and defect in membrane ionic conductances or transport mechanism.

1.3.1 Defect in GABA neurotransmitter

Many studies have revealed the involvement of altered GABA mediated inhibition in animal models of epilepsy. In some experimentally induced epileptic animals, GABA concentration, glutamic acid decarboxylase (GAD) activity and GABA binding were markedly decreased and thus probably contributed substantially to the epileptic discharges (Ribak et al., 1979; Ribak, 1983; Pitkanen et al., 1987; Horton, 1991). A reduction in GABA function is associated with lowered seizure threshold and spontaneous convulsions, whereas enhancing GABA-mediated inhibition is often associated with an increase seizure threshold (Horton, 1991).

1.3.2 Enhanced activity of excitatory neurotransmitters

Glutamate is the principal excitatory neurotransmitter in the brain and as such, it inevitably plays a role in the initiation and spread of seizure activity (Meldrum, 1994). Among various sub-types of glutamate receptors, it appears that N-methyl-D-aspartate (NMDA) receptors are the most likely candidates for a role in epilepsy as antagonists for this receptor have been shown to be an effective anticonvulsant in animal model (Meldrum et al., 1989). There are two approaches based on an inhibition of excitatory neurotransmitter which appear to be significant in the suppression of seizures. One is to act postsynaptically on receptors to decrease the excitation induced by glutamate and another is to decrease synaptic release of glutamate and aspartate (Meldrum, 1991).

A recent study of pilocarpine-induced limbic convulsions in rodents reveals an increase in the extracellular concentration of aspartate in the hippocampus before seizure onset, but not during seizures (Millan, Chapman and Meldrum, 1993). An extensive study employing chronically implanted bilateral hippocampal electrodes and microdialysis probes shows a marked increase in extracellular glutamate concentration directly before electrographic seizure onset on the side of focal origin of the seizure (During and Spencer, 1993). However, some microdialysis studies in animal models with brief or prolonged seizures have provided somewhat perplexing data, with frequently no evident change in extracellular glutamate concentration even during prolonged convulsions induced by systemic convulsant agents.

1.3.3 Defect in membrane ionic conductances or transport mechanism

The efficacies of various ionic channels depend on extracellular and intracellular ion concentrations. Potassium conductances are much less effective in reducing cell excitability under conditions of increased extracellular potassium, because there is a much smaller driving force for potassium efflux. Further, increased extracellular potassium itself causes cell depolarization and increase cell excitability (Schwartzkroin, 1993).

The voltage-dependent conductance in terminal membrane is critical for the release of neurotransmitter. Although little is known about the specific channels in the terminals of central nervous system cells, calcium influx (or rise in intracellular calcium level) is essential for transmitter release (Zucker and Lando, 1986).

It was found that Glia cell passively transferred excess K^+ away from a region of excitation and maintain extracellular homeostasis. They could play a role in preventing the onset of seizure activity locally as well as excessive neuronal depolarization in epileptogenesis (Goldring, 1978).

2. Classification of epilepsy

The classification of epilepsy is complicated and can be based on the etiology, age of onset, clinical seizure, electroencephalography (EEG) findings or prognosis. A revised classification of individual seizure types was accepted in 1982 by the General Assembly of the International Leaque Against Epilepsy (ILAE) (Table 1). It is a guiding useful tool for making decisions on how to treat

epilepsy and how to choose among the available antiepileptic drug (Fukuzako and Izumi, 1991). In general, the neurologists use the clinical symptoms and the EEG patterns to classify epileptic patients into two categories: partial seizures and generalized seizure (Rall and Schleifer, 1990).

Partial seizures have clinical or EEG evidence of a local onset. The abnormal discharge usually arises from a portion of one hemisphere and may spread to other parts of the brain during a seizure (Fukuzako and Izumi, 1991). Generalized seizures have no evidence of localized onset, the abnormal electrical discharges appear simultaneously over the entire cerebral cortex. Many epileptologists infer from this discharge pattern that wide areas of cortex are driven synchronously by diffuse projections from deep cerebral structures, probably in the thalamus or reticular system (Alldredge, 1992).

However, there are other types of seizures that cannot be classified as described due to their special characteristics as follows:

Infantile spasms : is an epileptic syndrome encompassing a group of attacks with remarkably diverse etiologies, in which most patients have the onset of seizure before the age of one year. The recurring attacks, trunk and extremities, are usually associated with mental retardation and hypsarrhythmia in the EEG (Porter, 1993).

Lennox-Gastaut syndrome : usually begins between the ages of 1 and 6 years, though rarely it may occur as 10 years of age or older. The etiologies are diverse. Most patients are mentally retarded. The most devastating seizures are

Table1

International Classification of Epileptic Seizures in 1981 (Fukuzako and Izumi, 1991)

I Partial seizuers

- A. Simple partial seizures
  1. With motor symptoms
  2. With somatosensory or special sensory symptoms
  3. With autonomic symptoms
  4. With psychic symptoms
- B. Complex partial seizures
  1. Simple partial onset followed by impairment of consciousness
     a. With no other features
     b. With features as in A.1-4
     c. With automatisms
  2. With impairment of consciousness at onset
     a. With no other features
     b. With features as in A.1-4
     c. With automatisms
- C. Partial seizures evolving to secondarily generalized seizures

II. Generalized seizures

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

III. Unclassified epileptic seizures

Modified from Commission on Clasification and Terminology of the International League Against Epilepsy.

atonic, the head may drop suddenly, or the patient may fall precipitously to the floor. The EEG commonly demonstrates continuous spike-and-wave abnormalities, usually at a rate which is less than 3 per second, and the complexes are often irregular and asymmetrical; multifocal spikes are also common (Porter, 1993).

Febrile convulsion : occurs in 2-5% of population (Hauser, 1981); the attacks are usually benign. They occur between 3 months and 5 years of age and are associated with fever but not intracranial infection (Annegers et al., 1987).

Neonatal seizure : in a newborn baby is likely to become symptomatic of epilepsy in later childhood. The common causes of neonatal seizure is congenital malformations, hypoxia, infections trauma, intracranial hemorrhage and various metabolic disorders (Fukuzako and Izumi, 1991).

Reflex epilepsy : occurs in response to a fixed and clearly recognized sensation or perception such as flickering light, noise, music, etc. Photosensitive epilepsy is the most common form of such epilepsy (Fukuzako and Izumi, 1991).

Status epilepsy : consists of a recurrent seizure without recovery of consciousness between attacks. There are three types of this epilepsy, convulsive status, nonconvulsive status, and continuous partial seizures (Fukuzako and Izumi, 1991).

3. Amino acid neurotransmitters

Amino acids have gained recognition as major neurotransmitter in the central nervous system (CNS). On the basis of neurophysiological studies, amino

acids have been separated into two general classes: excitatory amino acids (glutamic acid, aspartic acid, cysteic acid, and homocysteic acid), which depolarize neurons in the mammalian CNS; and inhibitory amino acids (GABA, glycine, taurine, and β-alanine), which hyperpolarize mammalian neurons in the mammalian CNS (Cooper, Bloom, and Roth, 1991).

3.1 Excitatory amino acid neurotransmitter

Glutamate and aspartate occur in uniquely high concentrations in the brain and that they can exert very powerful stimulatory effects on neuronal activity. These appear to play important roles in the initiation, spread and maintenance of epileptic activity, neurodegenerative diseases and cerebral ischemia (Faden, Ellison and Noble, 1990; Meldrum, 1994).

Glutamate is the predominant excitatory neurotransmitter in the brain and spinal cord, besides being the essential immediate precursor for the synthesis of GABA in the CNS and an important intermediate in neuronal metabolism. The postsynaptic action of glutamate when applied to neurones is always excitatory, mediated through either an ionotropic or metabotropic action (Greenamyre and Porter, 1994).

Glutamate is produced from α-ketoglutarate by glutamic acid dehydrogenase, from glutamine by glutaminase, from ornithine by ornithine aminotransferase via glutamate semialdehyde, and from proline by proline oxidase with subsequent oxidation of the intermediate, Δ-pyrrolin-5-carboxylic acid (McGeer, Eccle, and McGeer, 1988; Browning, 1991). In addition, transamination of α-ketoglutarate and oxaloacetate to glutamate and aspartate respectively can also

be achieved with the help of the enzyme aspartate aminotransferase (Asp-T), also known as glutamic-oxaloacetic transaminase (GOT) and glutamic-aspartic transaminase (McGeer et al., 1988).

Excitatory amino acid receptors

Classes of excitatory amino acid receptors have been expanded to at least five different types in the CNS, each displaying distinct physiological characteristics. Three of these receptors have been defined by the depolarizing excitatory actions of selected agonist NMDA, kinate and quisqualate or alpha-amino-3-hydroxy-5-methyl-ioxyzole-4-propionic acid (AMPA) receptor. A fourth, the L-2 amino-4-phosphonobutyrate (AP4) receptor appears to represent an inhibitory autoreceptor. The fifth receptor, activated by trans-I-aminocyclopentane-I-3-dicarboxylic acid (ACPD) modifies inositol phosphate (IP) metabolism and has been called a metabotrophic receptor (Cooper et al., 1991).

The possible role of NMDA receptor-gated ion channels (NMDA channel) is necessary for epilepsy. Superficially there is a marked similarity between the NMDA (Figure 1) and the GABA receptor complexes (Figure 2). Both have multiple binding sites which modulate the affinity of the endogenous ligand for its receptor. The NMDA channel allows the influx of Na^+ and Ca^{2+} and the efflux of K^+ and within this channel there is a binding site for Mg^{2+} which at resting potentials blocks the channel. However depolarization of the post synaptic neurone by, for example, stimulation of the quisqualate or AMPA receptor removes the Mg^{2+} block on the NMDA channel, the block is thus voltage dependent (Davis and Richens, 1993).

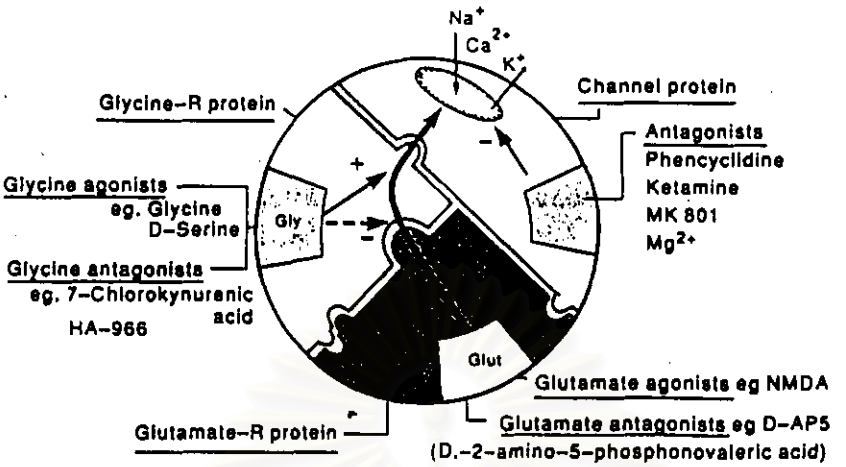

Figure 1. Glutamate (NMDA) receptor complex

(Davies and Richens, 1998)

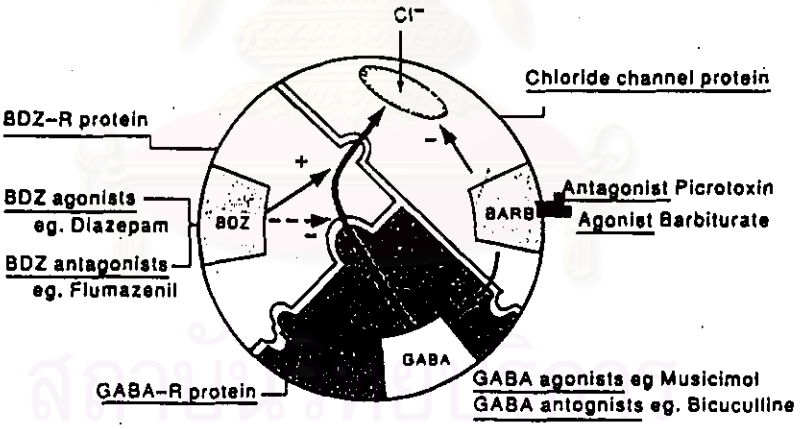

Figure 2. $GABA_A$ receptor complex

(Davies and Richens, 1998)

3.2 Inhibitory amino acid neurotransmitter

3.2.1 GABA

GABA is the major inhibitory neurotransmitter in the CNS and is found in all brain regions. It has been estimated to be the transmitter at 30% of all the synapses in the brain. There are two types of inhibitory mechanisms in the CNS, presynaptic and postsynaptic. In the former, GABA acts on a presynaptic terminal of an excitatory neurone to prevent release of transmitter; this form of inhibition is found predominantly in the spinal cord. Postsynaptic inhibition is the main inhibitory mechanism found in the brain and it is at this site that most of antiepileptic drug exert their action (Davies and Richens, 1993).

Distribution

GABA is found in high concentration, μmoles/g rather than nmoles/g, in the brain and spinal cord. Interestingly, the brain also contains large amount of glutamic acid (8-13 nmoles/g) which is the main precursor of GABA and itself a neurotransmitter candidate (Cooper et al., 1991).

The ubiquitous distribution of GABA in the brain can account for its utilization as a neurotransmitter in both interneurones and in long-axoned tracts. The interneurones of the cerebral cortex, such as small basket cell and the chandelier cell, are responsible for regulating the output of cortex and also the activity of the association fibres. The major long-axoned tracts which use GABA as a neurotransmitter are those from the hippocampus to the cortex (Davies and Richens, 1993).

GABA does not easily penetrate the blood brain barrier. It is difficult if not impossible to increase the brain concentrations of GABA by peripheral administration, unless one alters the blood brain barrier. Some investigators have tried to circumvent this problem by administration of GABA-lactam (2-pyrrolidinone) to animals in the hope that this less polar and more lipid soluble compound would penetrate more easily into the brain and be hydrolyzed to yield GABA (Cooper et al., 1991).

Synthesis and degradation

The major source of GABA in the CNS is most probably glucose entering the tricarboxylic acid (Krebs) cycle via pyruvate and subsequent production of α-ketoglutarate (Figure 3). The transamination of α -ketoglutarate produces L-glutamic acid (glutamate), the immediate precursor of GABA. Fundamental to GABA synthesis is so-called GABA shunt which operates within the terminal of the neuron.

GABA is formed by decarboxylation of L-glutamic acid by enzyme glutamic acid decarboxylase (GAD) which is found only in neurones and this decarboxylation is probably the rate-limiting step in the synthesis of GABA. The next reaction in the shunt is the transamination of GABA to succinic semialdehyde via GABA-transaminase (GABA-T). However, this can only take place in the presence of α-ketoglutarate to accept the amine group that is produced. This ensures that a molecule of glutamate is synthesized to take the place of the destroyed molecule of GABA. The succinate semialdehyde formed from GABA is oxidized to succinate and this enters the tricarboxylic acid cycle (Davies and Richens, 1993).

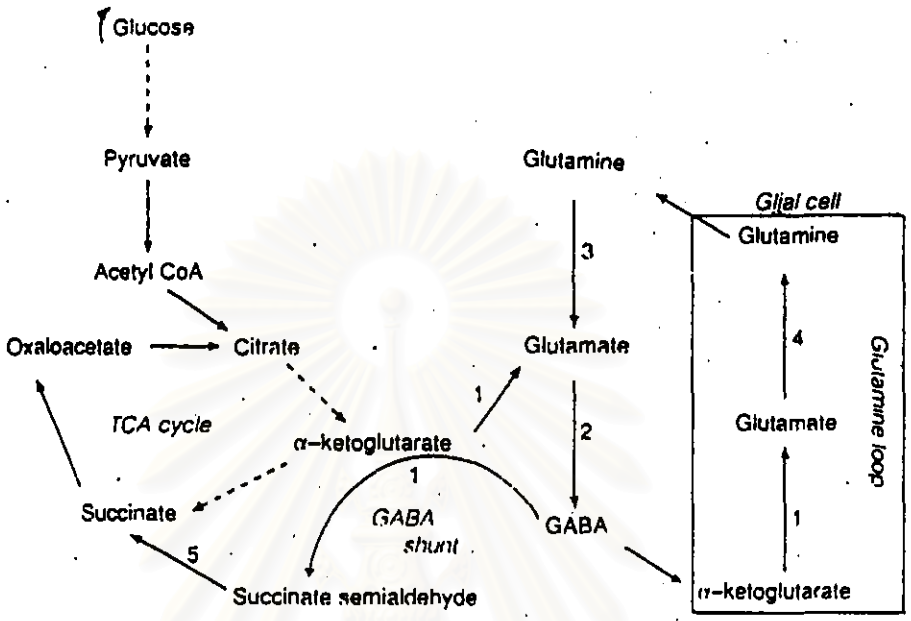

Figure 3. Metabolic pathway for the synthesis and degradation of GABA and glutamate. 1:GABA - transaminase (pyridoxal phosphate required as co-factor); 2:Glutamic acid decarboxylase; 3:Glutaminase; 4:Glutamine synthetase; 5:Succinic semialdehyde dehydrogenase.

GABA receptor

GABA receptors when occupied by the endogenous ligand invariably produce hyperpolarization in the postsynaptic neurone. There are two types of receptor designated as $GABA_A$ and $GABA_B$ (Matsumoto, 1989). $GABA_A$

receptors are the classical bicuculline-sensitive receptors and it is these which are important in the treatment of epilepsy while the $GABA_B$ receptors are activated by baclofen (Bowery, Hill and Moratalla, 1989). Synaptically stimulation of these results in either pre-or postsynaptic inhibition.

$GABA_A$receptors originally described as the $GABA_A$/benzodiazepine receptor complex consists of two α and two β subunits (Levitan et al., 1988). However, recent reports indicate that there is a third type of subunit (γ) in the $GABA_A$/benzodiazepine complex in the brain (Olsen and Tobin,1990). Activation of $GABA_A$ receptors (Figure 2) opens the chloride channel in the GABA/benzodiazepine complex and this allows an influx of Cl^- which leads to hyperpolarization of the postsynaptic neurone consequently an inhibition of firing. As this mechanism involves ionic fluxes, it has been termed ionotropic transmission (McGeer et al., 1987).

$GABA_B$ receptor was found at lower levels in the CNS than $GABA_A$ receptor. This receptor has been classified as metabotropic receptor because the transduction mechanism at these sites involved a second messenger resulting in metabolic changes. A linkage of the $GABA_B$ receptor to a G protein (s) is the most probable transduction process (Wojcik, Paez and Ulivi, 1989). Activation of $GABA_B$ receptors leads to a decrease in Ca^{2+} conductance (infux) and/or an increase in K^+ conductance. The latter, producing an efflux of K^+, would lead to hyperpolarization of the neurone. Either of this possibilities would decrease the release of neurotransmitters (Browning, 1991).

3.2.2 Glycine

Glycine is the simplest amino acid found in all mammalian body fluids and tissue proteins in substantial amounts. It is believed to function as a neurotransmitter in spinal cord. Furthermore it has been suggested that this amino acid may play a similar role in more rostral portion of the CNS as well as in the retina (Cooper et al., 1991).

Distribution

In comparison to other amino acids, glycine is found in relatively high concentrations in the spinal cord, displaying higher concentration in spinal gray matter than in the spinal white matter (Cooper et al., 1991).

Synthesis and degradation

Glycine is synthesized from glucose via glycolytic pathway to produce 3-phosphoglycerate and 3-phosphoserine which is then converted to glycine by a reversible folate-dependent reaction catalyzed by the enzyme serine hydroxymethyltransferase (SHMT). Glycine can also be formed from glycoxylate by transamination (Cooper, Bloom et al., 1991; Browning, 1991).

Glycine receptors

Glycine receptors can be classified into two subtypes according to strychnine sensitivity. The strychnine-sensitive glycine receptor appears to exist in a

macromolccular complex which consists of the glycine recognition site, chloride channel and strychine binding sites (Davidoff, 1983; Browning, 1991). Activation of strychine-sensitive glycine receptor, like the $GABA_A$ receptor, cause an increase in chloride conductance which usually results in hyperpolarization and inhibition of postsynaptic membrane, and this effect can be antagonisted by strychnine (Davidoff, 1983; McGeer et al., 1988; Browning, 1991). The strychnine-insensitive glycine receptors are linked to the NMDA excitatory amino acid receptor. When compared with the effects of benzodiazepine on the GABA receptor, the enhancement of NMDA responses observed with glycine is much greater, suggesting that the main effect of glycine is to prevent desensitization of the NMDA receptor during prolong exposure to agonists. The endogenous strychnine-insensitive glycine receptor agonist appear to be the tryptophan metabolite and 7-chloro kynurenic acid and which is relatively more selective and more potent (Cooper et al., 1991; Browning, 1991).

4. Drugs for the treatment of epilepsy

The best treatment to control epilepsy must be selected on the basis of proper diagnosis and classification of epilepsy (Fukuzako and Isumi, 1991). Monotherapy should be recommended in a newly diagnosed epileptic patient and polytherapy is needed when patient is not seizure free with the existing monotherapy. Guidelines for planning a rational polypharmacy regimen are combined drugs with different mechanisms of action for synergism of anticonvulsant effects, avoidance of drugs with similar adverse effects or drugs exhibiting significant enzyme inhibition or enzyme induction (Wider, 1995).

4.1 Conventional drugs

The drugs of choice for simple and complex partial and tonic-clonic seizures are phenytoin and carbamazepine. Absence myoclonic, and primary generalized tonic-clonic seizures respond best to valproic acid (Figure 4). Ethosuximide, a highly specific antiepileptic drug, is the drug of choice for absence seizure only. Atonic seizures respond poorly to most conventional therapies. (Mattson et al., 1992)

The drugs of choice for symptomatic and secondary epileptic syndromes, such as simple or complex partial and secondary generalized seizures, are phenytoin and carbamazepine. For primary generalized epilepsies, including simple absence seizures and juvenile myoclonic epilepsy, ethosuximide and valproic acid are the agents of choice. These epileptic syndromes seem to respond better to pharmacologic treatment than do the symptomatic or secondary epilepsies. Epilepsies characterized by drop attacks, tonic seizures, atypical absence,infantile spasms, West's syndrome and epilepsies associated with psychomotor retardation typically respond poorly to conventional antiepileptic drugs and other treatments. Lennox- Gastaut syndrome responds to valproic acid, although the response is not considered satisfactory (Figure 5) (Wilder, 1995).

4.2 New drugs

Three new antiepileptic drgus are now clinically available: felbamate, gabapentin and lamotrigine. These drugs have all undergone controlled clinical trials for efficacy and safety in patient with predominantly simple, complex

partial and generalized tonic-clonic seizures (Figure 6). All of new antiepileptic drugs have demonstrated efficacy in double-blind and dose-ranging studies. Few controlled clinical trials have been conducted in patients with absence and myoclonic seizures, although trials currently are underway (Wilder, 1995). Felbamate has demonstrated efficacy as monotherapy in the treatment of partial seizures with or without secondary generalization in adults, as well as in the treatment of partial and generalized seizures associated with Lennox-Gastout syndrome in children (Faught et al., 1993; Sachdeo et al., 1992 and Bourgeois, 1993). Lamotrigine apperars to have a broad spectrum of antiepileptic activity. It has demonstrated efficacy as add-on therapy in the treatment of adults who have partial seizures with or without secondary generalized tonic-clonic seizures (Matsuo et al., 1993 and Messenheimer et al., 1994). and in patients with Lennox-Gastaut syndrome (Oller, Russi and Daurella, 1991). In two recent studies, lamotrigine monotherapy demonstrated efficacy in patients with newly diagnosed generalized tonic-clonic seizures or recurrent partial or generalized tonic-clonic seizures (Steiner and Yuen, 1994).

4.3 Development of antiepileptic drug

About 30% of patient with epilepsy remains refractory to medical management despite optimal use of conventional antiepileptic drugs. Seizure control for many patients is often achieved at the expense of adverse drug effects such as depression, behavioral changes, slurred speech, tremors, and cosmetic problems including weight gain, hair loss, gum overgrowth, and coarsening of facial features. The need of new drugs is obvious; it may be possible to overcome this dilemma by developing antiepileptic drug with aims to increase potency and decrease drug adverse effect (Wilder, 1995).

In classical drug develoment for epilepsy, anticonvulsant activity was fortuitously discoverd from a new chemical substances or developing improved derivatives of the existing antiepileptic drugs (Bialer et al., 1994).

Recently, understanding of the major processes involved in epilepsy helps to define the potential mechanisms of antiepileptic drugs as following: (1) changes in voltage-regulated ion channels in neuronal membranes that leading to excessive depolarization or excessive action potential firing, (2) decreased inhibition (GABA receptor/channel complex) subsequently allowing excessive neuronal firing (3) increased excitation mediated through the NMDA receptor and (4) changes in extracellular concentrations of potassium and calcium (Graves and Leppik, 1991).

In general, antiepileptic drugs act through multiple mechanisms with multiple sites of action. For many of the new antiepileptic drug, clear-cut mechanism of action remain to be demonstrated. Antiepileptic drugs may disrupt one or more mechanisms involved in the genesis and spread of epileptic discharges, as following:

Felbamate : is thought to act as a blocker of glycine at the NMDA receptor. It blocks repetitive neuronal firing, perhaps by prolonging the inactive phase of sodium channels. It may enhance chloride entry into the chloride channel, resulting in increasing inhibition (Wilder, 1995)

Gabapentin : is an analogue of GABA. It has higher lipophilicity than the naturally occuring inhibitory neurotransmitter, which permits gabapentin to penetrate the blood-brain barrier more readily (Sivenius et al.,1991;Graves and Leppik,1991). Gabapentin binds to neuronal membranes, however, its mechanism of

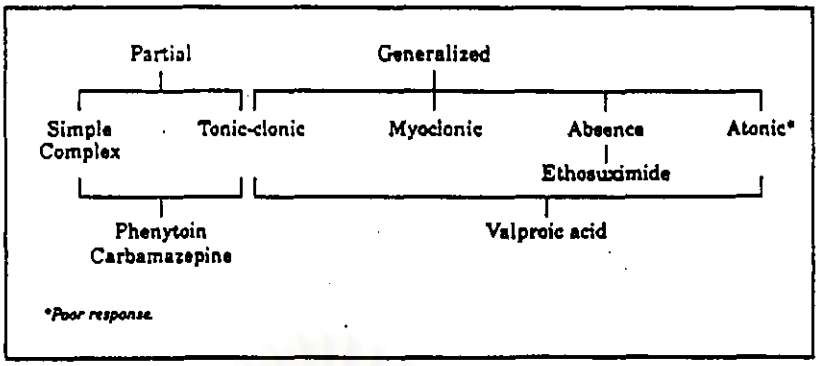

Figure 4. Specificity of conventional antiepileptic drugs in the treatment of partial and generalized seizures.

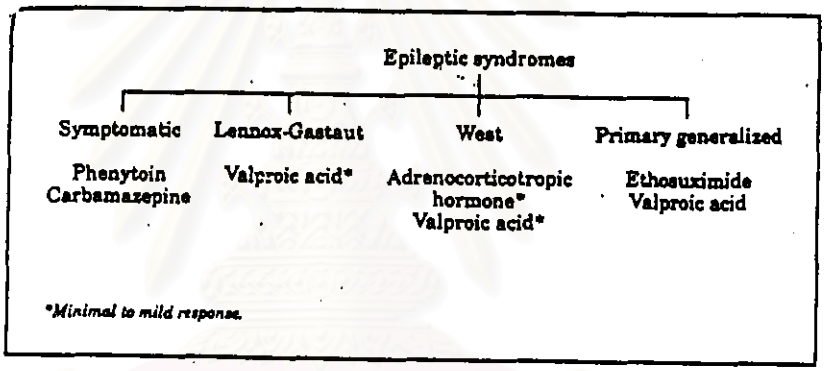

Figure 5. Specificity of conventional antiepileptic drugs in the treatment of epileptic syndromes.

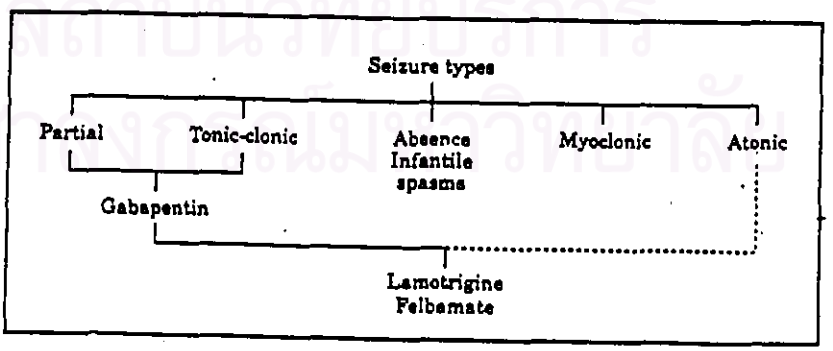

Figure 6. Specificity of new antiepileptic drugs in the treatment of partial and generalized seizures.

action is unknown. A delayed block of sodium channels occurs some hours after administration, but whether this is a primary or secondary action is not yet known (Graves and Leppik,1991).

Lamotrigine : studies suggest that lamotrigine may inhibit excitatory glutamate and aspartate release, and its effects a dose-dependent blockade of sodium channels and prevents repetitive firing(Leach, Marden and Miller,1986). Lamotrigine blocks visually evoked afterdischarge, which is perhaps indicative of a broad spectrum of activity (Lamb and Miller,1985).

Valproic acid

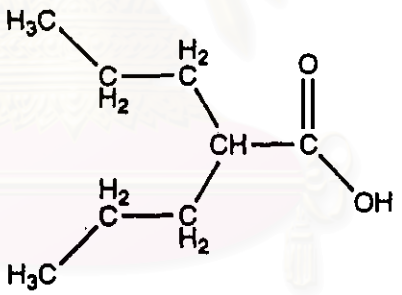

Figure 7. Structural formula of valproic acid

Valproic acid (VPA; 2-propylpentanoic acid) is a branched-chain fatty acid, and differs structurally from other antiepileptic drugs due to its lack of a nitrogen molecule or a heterocyclic moiety (Figure 7).

VPA was introduced as the 16th primary antiepileptic drug marketed and licensed for use as an anticonvulsant in the United States in 1978. The drugs is available in several forms, including the parent compound, its sodium and amide derivative as well as a combination of the parent compound and its sodium salt. In addition, valproic acid and sodium valproate are available in capsule, tablet, enteric-coated tablet, liquid, intravenous, suppository and controlled-release formulations (Davis, Peters and Mctavish, 1994).

1.1 Mechanism of action

1.1.1 Effects on membrane

VPA appears to reduce sustained repetitive firing through its influence on sodium and potassium conductance. When high concentrations (50 to 30 m M/L) of the drug were applied to isolated aplysia neurones, an increase in membrane potassium conductance developed, leading to an increase in membrane potential (Slater and Johnston, 1978). VPA has been noted to protect mice against convulsions produced by potassium channel blockers (Moore et al., 1984).

1.1.2 Effects on GABA

The studies in rats suggested that antiepileptic effect of VPA was resulted from elevated brain GABA levels resulting from inhibition of the catabolic enzyme GABA-T (Godin et at., 1969). VPA has also demonstrated activation of GAD (Loscher, 1981; Phillips and Fowler, 1982), a major enzyme in GABA synthesis and strong inhibition of succinic semialdehyde dehydogenase an

enzyme in the GABA degradation pathway (Van der Laan, De boer and Bruinvels, 1979; Zeise, Lasparow and Zieglgansberger, 1991).

1.1.3 Effects on other amino acid neurotransmitters

There are evidences suggesting that valproic acid reduces neurotransmission mediated by excitatory amino acids such as aspartic acid, glutamic acid and γ-hydroxybutyric acid (Chapman et al., 1982; Vayer, Cash and Maitre, 1988).

1.2 Adverse drug action and interaction

Common side effect of valproate therapy was gastrointestinal effects (anorexia, indigestion, heartburn and nausea) rather than neurological effects (Davis et al., 1994). In animals, VPA exhibited two major side effects namely teratogenicity and hepatotoxicity (Jeavons, 1982).

In human, an estimated risk of 1-2% for neural tube defects, predominatly spina bifida aperta, with maternal use of VPA therapy has been reported (Dreifuss and Langer,1988; Davis et al., 1994). Although the precise biochemical mechanism for the teratogenic effects of VPA and other antiepileptic drugs is unknown, it has been suggested that alter folate metabolism and/or interferance with folate metabolism by antiepileptic drugs may be partly responsive for the malformation observed (Davis et al., 1994). The studies in rats (Sugimoto et al., 1987) and rat hepatocytes cultures (Jeavons, 1982) indicated that VPA exhibited a dose-related hepatotoxicity. Several possible biochemical mechanisms responsible for the hepatotoxicity have been proposed, and disputed. However, certain evidence

suggests that δ-dehydrogenation derivative 4-En-valproic acid is responsible for the effect observed (Eadie et al., 1990).

Management of epilepsy often requires concomitant use of more than one antiepileptic drug, therefore, the potential for clinically significant pharmacokinetic and pharmacodynamic interactions between these drugs must be considered. VPA is known to inhibit hepatic enzymes and would therefore be expected to increase the plasma concentration of other antiepileptic drug. Steady-state plasma of valproic acid concentrations was decreased during coadministration of the drug with hepatic enzyme-inducing agent such as carbamazepine, phenytoin, phenobarbital or primidone. These drugs increase the intrinsic clearance and decrease plasma values of valproic acid (Levy and Koch, 1982). The dosage of valproic acid has to be increased by 5 to 10 mg/kg/day when used in combination with other drugs that induce liver enzyme activity (Davis et al., 1994).

In addition, pharmacokinetic interactions between VPA and non-antiepileptic drugs, may potentiate the CNS depressant effects of alcohol and other CNS depressants (Davis et al., 1994). In contrast with other antiepileptic drugs, VPA does not decrease the effect of oral contraceptives (Crawford et al., 1986; Perucca et al., 1984). Salicylates have been shown to elevate both total and unbound plasma valproic acid concentration by decreasing valproic acid metabolism and displacing it from plasma albumin binding sites (Abbott et al., 1986; Goulden et al., 1987; Orr et al., 1982).

N-(2-Propylpentanoyl) Urea (VPU)

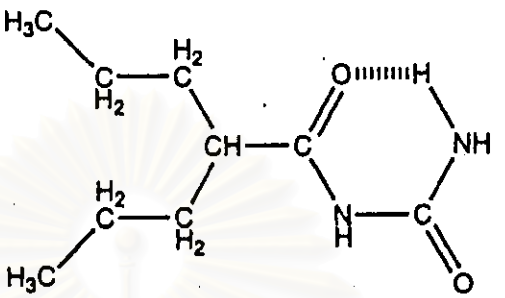

Figure 8. Structural formula of N-(2-Propylpentanoyl) Urea

N-(2-Propylpentanoyl) Urea (VPU) is a new synthetic chemical which was synthesized by Boonardt Saisorn and co-worker (1993). The structure of VPU modeled partially on barbiturate ring and VPA in the same molecule (Figure 8).

Previous studies by Thongchai Sooksawate (1995) have demonstrated anticonvulsant activity of VPU in maximal electroshock (MES) test as well as convulsion induced by pentylenetetrazole (PTZ) and bicuculline, however, it is rather ineffective in strychnine test. In comparison to VPA, VPU was relatively equipotent in bicuculline test but exerted higher potency in MES and PTZ tests. Furthermore, VPU appears to possess rather low side effects as predicted from its minor effects on locomotor activity and potentiation of barbiturate sleeping time.

In brain microdialysis studies, VPU decreased the level of cortical excitatory (aspartate and glutamate) and inhibitory (glycine and GABA) amino acid

neurotransmitter. The depression was greatest on glutamate and least on glycine. These may account for the anticonvulsant activity observed. However, a strong synergistic effect of VPU with pentobarbital, the anesthetic used, is questionable and has to be clarified in awake rats. Additional studies addressing this question as well as other pharmacological and toxicological profiles of this compound which are still lacking should be further undertaken. Thus, this investigation was aimed to determine :

1. Anticonvulsant efficacy and neurotoxicity of VPU in relation to time.
2. Degradation of VPU by liver and brain homogenates.
3. Effect of VPU on the level of excitatory and inhibitory amino acid neurotransmitters in the cerebral cortex of awake rats.