Chapter I

Introduction

Epilepsy

Epilepsy describes a condition in which a person has recurrent seizures due to a chronic, underlying process. This definition implies that a person with a single seizure, or recurrent seizures due to correctable or avoidable circumstances, does not necessarily have epilepsy. Epilepsy refers to a clinical phenomenon rather than a single disease entity, since there are many forms and causes of epilepsy. However among the many causes of epilepsy there are various epilepsy syndromes in which the clinical and pathologic characteristics are distinctive and suggest a specific underlying etiology. (Lowenstein, 1998).

The characteristic in epilepsy is the seizure, which is a paroxysmal event due to abnormal, excessive, hypersynchronous discharges of a set of neurons in the brain. The clinical manifestation consists of sudden and transitory abnormal phenomena which may include alterations of consciousness, motor, sensory, autonomic or psychic events. (Hopkins and Shorvon, 1995: Lowenstein, 1998)

Approximately 1% of the world's population has epilepsy, then about 50 million persons worldwide suffer from this disorder. Although standard therapy permits control of seizure in 80% of these patients, millions (500,000 people in the USA alone) have uncontrolled epilepsy. (Porter 1993; Porter and Meldrum, 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).

1. Etiology

Epileptic seizures are produced by abnormal discharges of neurons which may be caused by pathological process which affects the brain. In a significant proportion of

cases, however, no cause can be determined; these are known as the idiopathic or cryptogenic epilepsies. Possible explanations for idiopathic epilepsy include as yet unexplained metabolic or biochemical abnormalities and microscopic lesions in the brain resulting from brain trauma during birth or other injury. The term symptomatic epilepsy indicates that a probable cause has been identified (Dhillon and Sander, 1996).

1.1 Causes according to age

The likely etiology of epilepsy depends upon the age of the patient, which is one of the most important factors determining both the incidence and likely causes of epilepsy.

During the neonatal period and early infancy, potential causes include hypoxia-ischemic encephalopathy, trauma, CNS infection, congenital CNS abnormalities and metabolic disorders. The idiopathic or inherited forms of benign neonatal convulsions are also seen during this time period.

In young children and adolescent idiopathic seizures account for the majority of the epilepsies although trauma and infection also play a role. In this age group, particularly in children aged between 6 months and 5 years, seizures may occur in association with febrile illness. These are usually short, generalized tonic – clonic convulsions which occur during the early phase of a febrile disease. They must be distinguished from seizures that are triggered by CNS infections which produce fever, such as meningitis or encephalitis. Unless febrile seizures are prolonged, focal, recurrent, or there is a background of neurological handicap, the prognosis is excellent, and it is unlikely that the child will develop epilepsy (Dhillon and Sander, 1996; Lowenstein, 1998).

The range of causes of adult – onset epilepsy is very wide. Both idiopathic epilepsy and epilepsy due to birth trauma may also begin in early adulthood.

Other important causes are head injury, alcohol abuse, brain tumors and cerebrovascular disease. Brain tumors are responsible for the development of epilepsy in up to a third of patients between the age of 30 to 50 years. Over the age of 50 years, cevebrovascular disease is the commonest cause of epilepsy and may be present in up to half of the patients (Dhillon and Sander, 1996).

1.2 Genetic cause

The etiology of seizures remains unknown in a substantial number of patients. Inherited susceptibility is likely to be an important etiology, although the role of genetic factors in epilepsy has been almost unexplored (Roger and Porter, 1991). Genetic factors appear to be most significant in patients with various primary epilepsies (Menks, 1990).

The genetic causes of a few epilepsy syndrome have recently been discovered. Myoclonic epilepsy with ragged red fibers syndrome is associated with a mutation of mitochondrial tRNA-lysine. Mutations in the cystation B gene may cause another form of progressive myoclonus epilepsy. A number of other epilepsy syndromes have been mapped to chromosomal locations. Epilepsy has been produced in transqenic mice having a wide range of genetically engineered mutations, suggesting that many potential genetic abnormalities can result in a change in the seizure threshold (Lowenstein, 1998).

2. Epileptogenesis

The morphological abnormalities associated with epilepsy do not explain how seizures develop or propagate. The hypothesis that single or small groups of "epileptic" neurons produce abnormal electrical activity and drive normal neurons into seizure activity is being challenged by concept concerned with the plasticity of normal neurons in adapting to changes in their environment (Trescher and Lesser, 1996).

2.1 Neuronal Plasticity

At the extremes, neuronal plasticity may be responsible for neuronal hyperexcitability and hypersynchrony, which are primary features of epileptiform activity. Neuronal plasticity has been shown in mesial temporal sclerosis and in experimental models of hippocampal injury after kindling (Trescher and Lesser, 1996).

When neurons are lost, there is reorganization or "sprouting" of surviving neurons in a way that affects the excitability of the network. Some of these changes can be seen in experimental models of prolonged electrical seizures or traumatic brain injury. Thus, an initial injury such as head injury may load to a very focal, confined region of structural change that causes local hyperexcitability leads to further structural changes that evolve over time until the focal lesion produces clinically evident seizures (Lowenstein, 1998).

Investigations of the mechanisms of epileptic activity are beginning to address the interplay of the intrinsic electrical properties of the neurons against a background of the activity of entire cell populations. The electrical behavior of the cells, which is central to the normal as well as the abnormal activity of the neurons, depends on ion conductances, primarily of sodium, potassium, calcium and chloride. The ion conductances are in turn dependent of the intra–and extracellular concentrations of these ions as well as the ionic flux across the cell membrane, which is controlled by a combination of energy–dependent pump, voltage–gated channel, and neurotransmitter–controlled channels (Trescher and Lesser, 1996).

2.2 Neurotransmitter Systems

For the majority of human epileptic syndromes, the principal hypothesis currently concern are a reduction of inhibitory synaptic activity or enhancement of excitatory synaptic activity. These defective synaptic action might be expected to trigger a seizure. The neurotransmitter mediating the bulk of synaptic transmission in

mammalian brain are amino acids, namely, γ-aminobutyric acid (GABA) and glutamate which are the principal inhibitory and excitatory neurotransmitters, respectively (Heinemann and Jones, 1990; McNamara, 1996).

Insights into mechanism of seizure suggest that enhancing GABA – mediated synaptic inhibition would reduce neuronal excitability and raise the seizure threshold (McNamara, 1996). Many studies have revealed the involvement of 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 markly decreased and thus probably contributed substantially to the epileptic discharges (Ribak et al., 1979; Ribak, 1983; Pitkanen et al., 1987; Horton, 1991).

Glutamate is the major excitatory neurotransmitter in the CNS. It activates several receptor subtypes, The *N* – methyl – *D* – aspartate (NMDA) receptor is an ionophore complex that mediates calcium flux. Antagonist of the NMDA receptor have antiepileptic activity, but several *in vitro* and *in vivo* studies suggest the NMDA receptor has a greater role in epileptogenesis, rather than in maintaining already developed seizures. The non – NMDA ionotropic receptors primarily mediate fast synaptic transmission through sodium and potassium flux. The role of these non – NMDA receptors in epilepsy is less clear but under active investigation. Other neurotransmitter systems may play a modulatory role in epileptogenesis as well (Trescher and Lesser, 1996).

3. Classification of seizures

An essential step in the evaluation and management of a patient with a seizure is to determine the type of seizure that has occurred. The importance of this cannot be over emphasized. Classifying the seizure is essential for focusing the diagnostic approach on particular etiologies, selecting the appropriate therapy, and providing potential prognosis (Lowenstein, 1998).

The most widely accepted classification of seizures used today is the classification of epileptic seizures developed by the International League Against Epilepsy (ILAE) in 1981 (Table 1). This system is based on the clinical features of seizure and associated electroencephalographic (EEG) findings. Other potentially distinctive features such as etiology or cellular substrate are not factored into the classification system, although this will probably change in the future as more is learned about the pathophysiologic mechanisms that underlie specific seizure types (Pedley, Scheuer and Walczak, 1995 ; Lowenstein, 1998).

The main characteristic that distinguishes the different categories of seizures is whether the seizure activity is partial or generalized. Partial seizures are those in which the seizure activity is restricted to discrete areas of the cerebral cortex. Generalized seizures involve diffuse regions of the brain simultaneously in a bilaterally symmetric fashion. As a rule, partial seizures are typically associated with structural abnormalities of the brain. In contrast, generalized seizures may result from cellular, biochemical, or structural abnormalities that have a more widespread distribution.

Other aspects of seizure classification and phenomenology continue to be debated among epileptologists, including the definition of altered consciousness, use of the term "partial" versus "focal", and the concept of aura.

Not all seizure types can be classified as partial of generalized. This appears to be especially true of seizures that occur in neonates (i.e., less than 1 month of age) and infants (younger than 1 year). The distinctive phenotypes of seizures at these early ages likely result, in part, from differences in neuronal function and connectivity in the immature versus mature CNS (Lowenstein, 1998).

4. Amino acid neurotransmitters

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

Table 1. International Classification of Epileptic Seizures in 1981

I. Partial seizures

- A. Simple partial seizures
  1. With motor symptoms
  2. With somatosensory or special sensory symptoms
  3. With automatic 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
- E. Atonic seizures

III. Unclassified epileptic seizures

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

have been separated into two general classes : excitatory amino acid, glutamate and aspartate, which depalarize neurons in the mammalian CNS; and inhibitory amino acids, GABA and, to a lesser extent, glycine, which hyperpolarize mammalian neurons (Cooper, Bloom and Roth, 1991).

4.1 Inhibitory Amino acids

γ - Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the CNS and is found in all brain regions. It may play a role in the physiopathogenesis of certain neurological conditions, including epilepsy. 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 neuron to prevent release of transmitters; 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).

Glycine is an inhibitory neurotransmitter in mammalian spinal cord. Like GABA, it inhibits neuronal firing by gating Cl^- channels but with a characteristically different phamacology (Olsen and Delorey, 1999).

GABA synthesis

GABA is present in high concentrations (millimolar) in many brain regions. Glucose is the principal precursor for GABA production *in vivo*, although pyruvate and other amino acids also can act as precursors. The first step in the GABA shunt is the transmination of α - ketoglutarate, formed from glucose metabolism in the Krebs cycle by GABA α - oxoglutarate transaminase (GABA – T) into L – glutamic acid. Glutamic acid decarboxylase (GAD) catalyzes the decarboxylation of glutamic acid to form GABA. GAD appears to be expressed only in cells that use GABA as a neurotransmitter. GAD serves as an excellent marker for GABAergic neurons in the CNS. GABA is metabolized by GABA – T to form succinic semialdehyde. To conserve the available

supply of GABA, this transmination generally occurs when the initial parent compound, α - ketoglutarate, is present to accept the amino group removed from GABA, reforming glutamic acid. Therefore, a molecule of GABA can be metabolized only if a molecule of precursor is formed. Succinic semialdehyde can be oxidized by succinic semialdehyde dehydrogenase (SSADH) into succinic acid and can then reenter the Krebs cycle.

GABA release into the synaptic cleft is stimulated by depolarization of presynaptic neurons. GABA diffuses across the cleft of the target receptors on the postsynaptic surface. The action of GABA at the synapse is terminated by reuptake into both presynaptic nerve terminals and surrounding glial cells. (Olsen and Delorey, 1999).

GABA Receptors

The actions of GABA are mediated by of least two distinct classes of receptors, $GABA_A$ and $GABA_B$. They differ in their pharmacological, electrophysiological and biochemical properties.

The principal postsynaptic receptor of synaptically released GABA is termed $GABA_A$ receptor, which is a member of a superfamily of ligand – gated ion channel receptors. Activation of the $GABA_A$ receptor leads to inhibition of the postsynaptic cell by increasing the flow of Cl^- ion into the cell, which tends to hyperpolarized the neuron. (McNamara, 1996).

$GABA_B$ receptor, which is a member of *G* – protein – linked receptor superfamily, can mediate both postsynaptic and presynaptic inhibition. Activation of $GABA_B$ receptor leads to a decrease in Ca^{2+} conductance (influx) and/or an increase in K^+ conductance. The latter, producing an efflux of K^+, would lead to hyperpolarization of the neuron. Either of this possibilities would decrease the release of neurotransmitters (Browning, 1991; Olsen and Delorey, 1999).

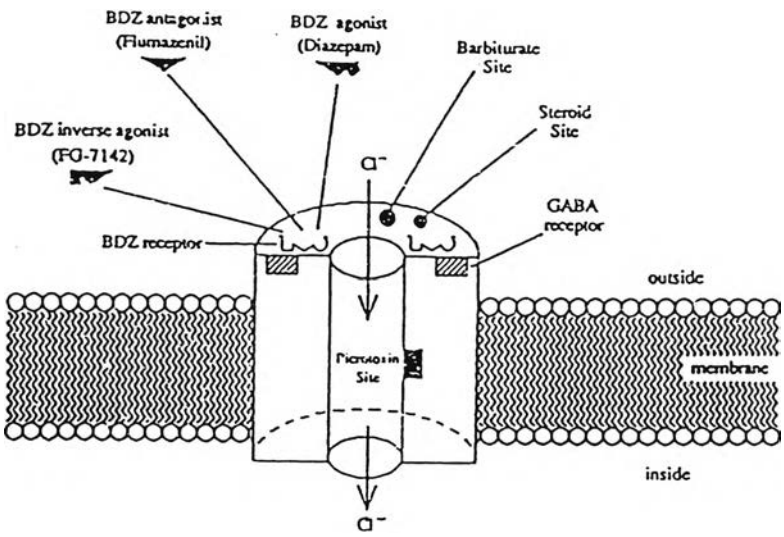

Figure 1. Schematic illustration of $GABA_A$ receptor complex.

4.2 Excitatory amino acid

Glutamate and aspartate occur in uniquely high concentrations in the brain. They exert powerful stimulatory effects on neuronal activity which play an important role in the initiation and spread of seizure activity. There is increasing evidence that an abnormality of excitatory amino acid (EAA) mediated neurotransmission may contribute to the epileptic phenomenon in various animal and human syndrome (Meldrum, 1996).

Glutamate is the predominant excitatory neurotransmitter in CNS, besides being the essential immediate precursor for the synthesis of GABA and 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 formed mainly from the Krebs cycle intermediate, α - ketoglutamate, by the action of GABA – aminotransferase (traver and Way, 1995).

Glutamate Receptors

Two main subtypes of glutamate receptors are ionotropic (receptors that are coupled directly to membrane ion channels) and metabotropic receptors (receptors that are coupled to G-protein and modulate intracellular secondary messenger such as cyclic neucleoside, inositol triphosphate and calcium). The ionotropic receptors are further classied as NMDA, receptor that produces a much slower response, and non-NMDA, AMPA and KA, which insensitive to the synthetic agonist NMDA (Lipton and Rosenberg, 1994).

Glutamate exerts its excitatory action via ligand – gated ion channels (NMDA and non – NMDA receptors) to increase sodium and calcium conductance. Glutamate also acts on neuronal and glial metabotropic receptors. Metabotropic receptors can be categorized in to 3 classes of receptors depending on pharmacological diversity and

coupling to second messenger systems. Activation of different classes of metabotropic receptors can result in both convulsant and anticonvulsant (Chapman, 1998).

5. Antiepileptic drugs

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).

5.1 Selection of antiepileptic drugs

For partial seizure, carbamazepine and phenytoin are drugs of first choice for the treatment of partial seizure, including those that secondarily generalize. Overall they have very similar efficacy, but differences in pharmacokinetics and toxicity are the main determinants for use in a given patient. Valproic acid is an effective alternative for some patients with partial seizures, especially when the seizures secondarily generalize.

Lamotrigine, gabapentin and phenobarbital are additional drugs currently used for the treatment of partial seizures with of without secondary generalization. (Pedley, Scheuer and Walczak, 1995 ; Lowenstein, 1998)

For generalized seizure, valproic acid is currently considered the best initial choice for the treatment of primarily generalized, tonic-clonic seizures, and

carbamazepine and phenytoin are suitable alternatives. Valproic acid is also particularly effective in absence, myoclonic, and atonic seizures and is therefore the drug of choice in patients with epilepsy syndromes having mixed seizure type. Ethosuximide remains the preferred drug for the treatment of uncomplicated absence seizures, but it is not effective against tonic-clonic or partial seizures. Clonazepam is an alternative for the treatment of myoclonic, atonic, and absence seizure, but it is not indicated for the treatment of most other seizure types (Table 2). Although approved for use in partial seizure disorders, lamotrigine is proving to be effective in epilepsy syndromes with mixed, generalized seizure types such as Juvenile myoclonic epilepsy and Lennox-Gastaut syndrome. (Lowenstein, 1998)

Table 2 Anitiepileptic Drugs of Choice

Focal-Onset Seizures*	Generalized Seizures			
	Generalized Tonic - Clonic	Absence	Myoclonic	Atonic
FIRST - LINE				
Carbamazepine Phenytoin Valproic acid	Valproic acid Carbamazepine Phenytoin	Ethosuximide Valproic acid	Valproic acid	Valproic acid
ALTERNATIVES				
Lamotrigine Gabapentin Phenobarbital Primidone	Phenobarbital Primidone	Acetazolamide Clonazepam Phenobarbital	Clonazepam Acetazolamide	Clonazepam

* Simple-partial, complex-partial, and secondarily generalized tonic-clonic seizures.

5.2 Mechanism of action of antiepileptic drugs

The principal mechanisms of action of antiepileptic drugs have been found to concern voltage-operated ion channels and inhibitory and excitatory synaptic function. Voltage-dependent Na^+ channels enter an inactive state following each action potential. Prolongation of this inactive state with a concomitant prolongation of refractoriness is thought to be the principal mechanism of action of phenytoin, carbamazepine and lamotrigine ; it may also contribute to the effects of phenobarbital, valproate, and topiramate. This phenomenon is linked to suppression of rapid repetitive firing in isolated neurons and protection against maximal electroshock in animals and focal seizures in man. (Porter and Meldrum, 1998)

A low-threshold Ca^{2+} current (the T-type Ca^{2+} current) has been found to govern oscillatory responses in thalamic neurons. Blocking of this current by ethosuximide or dimethadione is thought to explain the effect of these compounds in absence seizures (White, 1997 ; Porter and Meldrum, 1998).

Effect on synaptic transmission have been sought for many antiepileptic drugs. Enhancement of GABA-mediated inhibition can be produced in many different ways, involving either direct action on the GABA receptor-chloride channel complex (as with benzodiazepines, barbiturates) or actions on the reuptake or metabolism of GABA (as with tiagabine and vigabatrin). This mechanism provides protection against generalized and focal seizures. (Porter and Meldrum, 1998)

Reduction of excitatory glutamatergic neurotransmission is potentially important ; AMPA receptor blockade probably contributes to the effect of phenobarbital and topiramate, NMDA receptor blockade to the effect of remacemide, an investigational drug (Porter and Meldrum, 1998).

New antiepileptic drugs are being sought not only by the screening tests noted above but also by more rational approaches. Compounds are sought that

they may act by one of three mechanism : 1) enhancement of GABAergic (inhibitory) transmission ; 2) diminution of excitatory (usually glutamatergic) transmission, or 3) modification of ionic conductances (Na^+, Ca^{2+} or K^+) (Trevor and Way, 1995 ; Porter and Meldrum 1998).

1) Gabapentin (GBP)

The chemical structure of GBP is a GABA molecule covalently bound to a lipophilic cyclohexane ring. GBP was designed to be a centrally active GABA agonist, its high lipid solubility aimed at facilitating its transfer across the blood-brain barrier.

GBP inhibits tonic hindlimb extension in the electroshock seizure model. Interestingly, GBP also inhibits clonic seizures induced by pentylenetetrazol. GBP appears to act by a novel mechanism ; it increases release of GABA by an unknown mechanism (Honmou el at., 1995). GBP has not been found consistently to reduce sustained repetitive firing of action potentials nor to significantly affect any Ca^{2+} channel current (Macdonald and Kelly, 1993 ; McNamara, 1996).

2) Vigabatrin (VGB)

VGB, a close chemical analogue of GABA, is a novel antiepileptic drug designed to control seizure by raising brain GABA concentrations. (Petroff et al., 1996) VGB is an irreversible inhibitor of GABA-T, the enzyme responsible for the degradation of GABA (Porter and Meldrum, 1998). It 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 (White, 1997).

3) Lamotrigine (LTG)

LTG's principal mechanism of action, like that of phenytoin, concerns sodium channels. The drug blocks sustained repetitive firing of spinal cord neurons *in vitro* (McNamara, 1996). Its primary action is to stabilize presynaptic neuronal membranes by blockade of voltage-dependent sodium channels. This blockade leads secondarily to reduced release of excitatory amino acids, such as glutamate and aspartate (Laurence et al., 1997).

4) Tiagabine (TGB)

TGB is a derivative of nipecotic acid and was rationally designed as an inhibitor of GABA uptake. Although other compounds with this mechanism proved too toxic, TGB has been successfully developed (Porter and Meldrum, 1998). The action of TGB on GABA uptake leads to increased synaptic concentrations 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 (White, 1997).

5) Felbamate (FBM)

The mechanism of action of FBM has not been clearly established. It was believed to posses multiple mechanisms of action. Several lines of evidence indicate that FBM inhibits the NMDA-evoked response by modulation glutamate receptor function through an action on glycine, a co-agonist of glutamate necessary for activation of the NMDA receptor. In addition FBM also enhances GABA-evoked chloride currents *in vivo* (Upton, 1994; White, 1997).

Although, FBM remains a mechanistically very interesting AED with an apparently broad anticonvulsant profile, its clinical utility has unfortunately been markedly limited by the serious hematologic and hepatic toxicity observed after its commercialization in the United States (White, 1997).

6) Topiramate (TPM)

TPM is a sulphamate-substitute monosacharide, which is structurally different from all other antiepileptic drugs. TPM blocks repetitive firing of cultured spinal cord neurons as do phenytoin and carbamazepine. Its mechanism of action, therefore, is likely to involve blocking of voltage-dependent sodium channels. TPM also appears to potentiate the inhibitory effect of GABA by acting on the $GABA_A$ receptor at a site different from the benzadiazepine or barbiturate sites. It also depresses the excitatory action of kainate on AMPA receptors. It is possible that all three of these actions contribute to TPM's anticonvulsant effect (Porter and Meldrum, 1998).

Valproic acid

Valproic acid (VPA ; 2-propylpentanoic acid ; Figure 2) was synthesized in 1882 by Burton. For many years the substance was widely used as an organic solvent. The antiepileptic properties of VPA were discovered by chance in 1963 by Meunier et al. Within 15 years the drug was marketed worldwide, and during the past decade it has attained a position as one of the major antiepileptic drugs against several seizure types. (Gram, 1990)

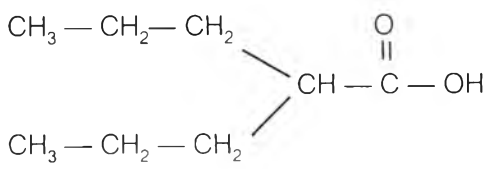

Figure 2 The chemical structure of valproic acid.

Chemistry

VPA is a branched-chain fatty or carboxylic acid that have antiepileptic activity; this activity appears to be greatest for carbon chain lengths of five to eight atoms. Branching and unsaturation do not significantly alter the drug's activity but may increase its lipophilicity, thereby increasing its duration of action. The amides and esters of VPA are also active antiepileptic agents (Porter and Meldrum, 1998).

VPA is highly effective against absence seizures and myoclonic seizure. In addition, VPA can be used either alone or in combination with other drugs for the treatment of generalized tonic-clonic epilepsy and for partial seizure with complex symptomatology (Craig , 1997).

Mechanism of action

Because VPA has such a wide spectrum of anticonvulsant activity, it is attractive to accept the view that the drug's clinical activity may relate to a combination of mechanisms (Rogawski and Porter, 1990).

1) Effects on GABA system

Whether or not VPA owes some of its broad spectrum of clinical efficacy to its presynaptic effects on GABA is controversial. When administered to mice, it is associate with increased GABA in the brain and at nerve terminals (Godin et al., 1969 ; Loscher, 1981a, b ; Poisson et al., 1984). However, attempts to correlate VPA's anticonvulsant effect with GABA concentrations have had inconsistent results (Kupferberg et al., 1975 ; Anlezark et al., 1976 ; Kerwin and Taberner, 1981 ; Nau and Loscher, 1982).

The mechanism by which VPA increases GABA levels is not well understood, but may involve effects on enzymatic synthesis of degradation of GABA.

VPA inhibits succinic semialdehyde dehydrogenase, an enzyme in the GABA degradation pathway (Van der Laan et al., 1979), but the relevance of this finding is undermined by the demonstration that near-total blockade of succinic semialdehyde dehydrogenase did not raise levels of whole-brain GABA. VPA also weakly inhibits GABA-T *in vitro* (Godin et al., 1969 ; Fowler et al., 1975 ; Loscher, 1980), but only at much higher levels than are clinically relevant in humans. GABA-T inhibition dose not occur *in vivo* after anticonvulsant doses of VPA (Nau and Loscher, 1982). VPA increases glutamic acid decarboxylase activity a major enzyme in GABA synthesis (Chapman et al., 1982 ; Phillips and Fowler, 1982), resulting in increased levels of GABA (Loscher and Nau, 1982). *In vivo* studies by Loscher (1989) demonstrated that VPA enhanced GABA synthesis and turnover only in the substantia nigra and striatum. The significance of these findings to VPA's overall mechanism of action remain unclear. (Waterhouse and Delorenzo, 1996).

Postsynaptic actions of VPA on GABA inhibition have also been studied. VPA enhances the inhibitory effects of GABA on neuronal activity *in vitro* (Macdonald and Bergey, 1979 ; Baldino and Geller, 1981 ; Preisendofer et al., 1987) and *in vivo* (Gent and Phillips, 1980). VPA also inhibits the binding of ^{3}H-dihydropicrotoxin to the GABA-receptor complex (Ticku and Davis, 1981). Because these postsynaptic effects only occur at VPA concentrations that exceed the clinical therapeutic range, a postsynaptic GABAergic effect of VPA is unlikely to contribute significantly to its clinical anticonvulsant actions (Waterhouse and Delorenzo, 1996).

2) Effects on membrane ion Channels

At therapeutically relevant concentration, VPA inhibits sustained repetitive firing induced by depolarization of mouse cortical or spinal cord neurons. The action is similar to that of both phenytoin and carbamazepine and appears to be mediated by a prolonged recovery of voltage-activated Na^{+} channels from inactivation. In neurons isolated from a distinct region, the nodose ganglion, VPA also produces small reductions of the low-threshold (T) Ca^{2+} current (Kelly et al., 1990) at clinically

relevant but slightly higher concentration than those that limit sustained repetitive firing; this effect on T currents is similar to that of ethosuximide in thalamic neurons (Coulter et al., 1989). Together, these actions of limiting sustained repetitive firing and reducing T currents may contribute to the effectiveness of VPA against partial and tonic-clonic seizures and absence seizures respectively (McNamara, 1996).

3) Effect on others amino acid neurotransmitters

VPA has been reported to reduce neurotransmission mediated by excitatory amino acids such as aspartic acid, glutamic acid and γ-hydroxybutyric acid (GHB). GHB has produced absence-like seizures in animals; therefore, reduction of its release may play a role in the efficacy of VPA in the treatment of absence seizures in humans (Davis, Peter and McTavish, 1994).

Phamacokinetic Properties

VPA is absorbed rapidly and completely after oral administration. Peak concentration in plasma is observed in 1 to 4 hours, although this can be delayed for several hours if the drug is administered in enteric-coated tablets or is ingested with meals (McNamara, 1996).

VPA has a pKa of 4.7 and is therefore virtually completely ionized at physiologic plasma pH. The drug is also 90% bound to plasma proteins, though the fraction bound is somewhat reduced at blood levels greater than 150 μg/ml. Since it is both highly ionized and highly protein-bound, its distribution is essentially confined to extracellular water, with a volume of distribution of approximately 0.2 liter/kg (Porter and Meldrum,1998). Although concentrations of VPA in CSF suggest equilibration with free drug in the blood, there is evidence for carrier-mediated transport of VPA both into and out of the CSF (McNamara, 1996).

In humans and animals, VPA is metabolized by several pathways. These include conjugation with UDP-glucuronic acid, β-oxidation and microsomal hydroxylation (ω, ω_1, ω_2 oxidation) (Zaccara et al., 1988). Glucuronidation is a major pathway resulting in several metabolites excreted in urine. β-oxidation reaction yields 2 metabolites, 2-propyl-2-pentanoic acid (2-ene-VPA) and 2-propyl 3-keto- pentanoic acid (3-keto VPA). ω, ω_1, ω_2 Oxidations, probably catalyzed by the microsomal CYP system, result in the formation of 3-OH, 4-OH and 5-OH metabolites. 5-OH VPA was further oxidized to 2-propyl glutaric (PGA). Moreover, in a minor amounts, two other unsaturated metabolites (4-ene-VPA and 2,4-diene-VPA) are also found (Figure 3) (Granneman et al., 1984; Nau and Loscher, 1984). 2-ene-VPA, a β - oxidation derivate, probably contributes to the anticonvulsant activity of VPA, while another metabolite, 4-ene-VPA, may be involved in both hepatic toxicity and embryotoxicity (Nau et al., 1984).

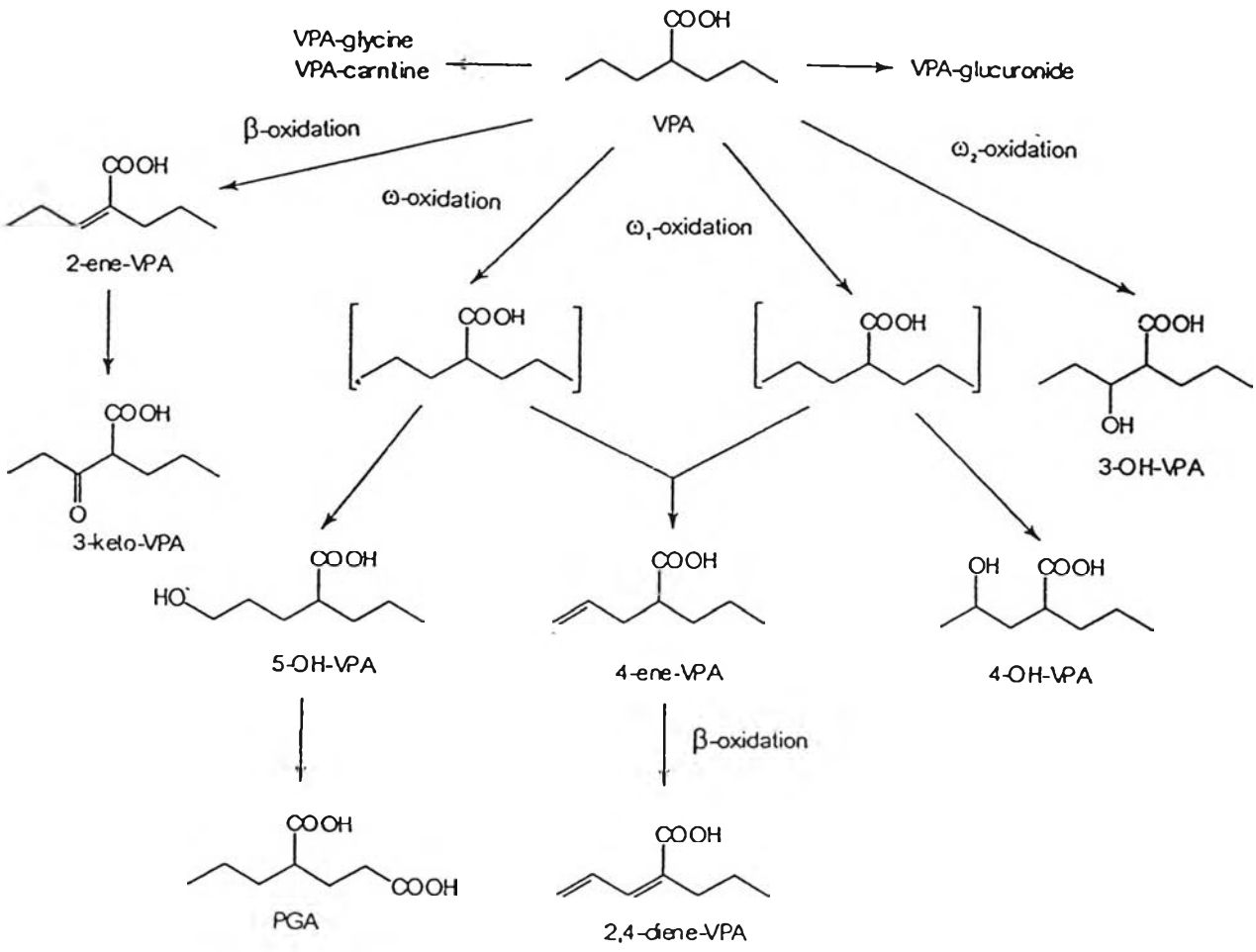

Figure 3.Metabolic pathways of VPA.

Toxicity

The most common dose-related adverse effects of VPA are transient gastrointestinal symptoms, including anorexia, nausea, vomiting and other gastrointestinal complaints such as abdominal pain and heart burn. The drug should be started gradually to avoid these symptoms. The availability of enteric-coated tablets of VPA has led to a significant decrease in the gastrointestinal side effect (Stringer, 1998). Effects on the CNS include sedation, ataxia and tremor. Sedation is uncommon with VPA alone but may be striking when VPA is added to phenobarbital. A fine tremor is frequently seen at higher levels. Other reversible adverse effects, seen in a small number of patients, include weight gain, increased appetite, and hair loss (Porter and Meldrum, 1994 ; McNamara, 1996).

Idiosyncratic reactions may be responsible for pancreatitis, haematological toxicity and hepatotoxicity. VPA also rarely causes reversible bone morrow suppression and hepatotoxicity and laboratory testing is required to monitor toxicity. This drug should generally be avoided in patients with preexisting bone marrow or liver disease. Irriversible, fatal hepatic failure appearing as an idiosyncratic rather than dose-related side effect is a relatively rare complication ; its risk is highest in children younger than 2 years old, especially those taking other antiepileptic drugs or with inborn error of metabolism. VPA therapy should therefore only be used in infants and young children when the benefits clearly exceed this risk (Lowenstein, 1998). The overall incidence of hepatic fatality was approximately 1 per 40,000 in monotherapy use. In high-risk intants, the overall risk of hepatic fatality was approximately 1 per 500. It has been suggested that in this high-risk group, VPA may interfere with some aspects of β-oxidation, though the role of the metabolites in the genesis of VPA hepatotoxicity has not been fully established. Metabolic analysis of VPA metabolites is not useful as a general routine screening method (Bruni, 1996).

Use of antiepileptic drugs during pregnancy is also associated with an increased risk of congenital malformations. An estimated risk of 1 to 2% for neural tube

defects, predominantly spina bifida aperta, with maternal use of VPA therapy has been reported. Although the precise biochemical mechanism for the teratogenic effects of VPA and other antiepileptic drugs is unknown, studies suggest that altered folate metabolism and/or interference with folate metabolism by antiepileptic drugs may be partly responsive for the malformations observed (Davis, Peter and McTavish, 1994).

Drug Interactions

The management of epilepsy often requires concomitant use of more than one antiepileptic drug, the potential for clinically significant pharmacokinetic and pharmacodynamic interactions between these drugs must be considered (Davis, Peter and McTavish, 1994).

Steady-state plasma VPA concentrations decrease during coadministration of the drug with hepatic enzyme-inducing agents such as carbamazepine, phenytoin, phenobarbital of primidone. These drugs increase the intrinsic clearance and decrease plasma half-life values of VPA, presumably by enzymatic induction of metabolism (Levy and Koch, 1982). The dosage of VPA may need to be increased by 5 to 10 mg/kg/day when used in combination with other drugs that induce liver enzyme activity (Davis, Peter and McTavish, 1994).

VPA is known to inhibit hepatic enzymes and would therefore be expected to increase the plasma concentrations of other antiepileptic drugs. Concentrations of phenobarbital in plasma rise by as much as 40% when VPA is given concurrently. The underlying mechanism probably involves inhibition of phenobarbital metabolism; its half-life is prolonged and urinary excretion of unchanged drug is increased. VPA also may inhibit the metabolism of phenytoin, but a change in its total concentration in plasma may not occur because of the simultaneous displacement of phenytoin from protein binding sites. Nevertheless, an increase in the concentration of free drug is possible (McNamara, 1996).

With regards to pharmacokinetic interaction with combinations of VPA and non-antiepileptic drugs, VPA may potentiate the CNS depressant effects of alcohol and other CNS depressants (Davis, Peter and McTavish, 1994).

VPA is one of the major antiepileptic drugs. While it has a broad antiepileptic spectrum of activity, two serious (although rare) side effects, teratogenicity and hepatotoxicity, have been associated with VPA therapy. Comparative analysis of the anticonvulsant potency and safety margin, utilizing the classical rodent models for anticonvulsant screening, shows that VPA is less potent than the other three major antiepileptics ; phenobarbital, phenytoin, and carbamazepine. Consequently, there is a substantial need to develop improved derivatives of VPA (Bialer et al., 1994; Levi, Yagen and Bialer, 1997).

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 humans VPD serves as a prodrug to VPA. Therefore, there is a need to develop stable VPD analogues. In the current study ,several novel derivatives of VPD were synthesized. Valproyl hydroxamic acid (VHA) (Figure 4) is one of the novel derivatives (Levi, Yagen and Bialer,1997).

Valproyl hydroxamic acid (VHA)

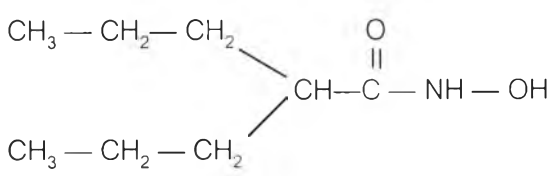

Figure 4 The chemical structure of valproyl hydroxamic acid.

In previous studies by Levi, Yagen and Bialer (1997), Valproyl hydroxamic acid (VHA) is a novel derivative of valpromide (VPD) which showed better anticonvulsant

activity than VPA, in maximal electroshock (MES) test, because of their greater intrinsic activity and not due to 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. In addition, it has been shown that unlike VPD, VHA was not biotransformed *in vivo* to VPA. However, additional studies in other pharmacological and toxicological profiles of this compound which are still lacking should be further undertaken.

In Thailand, VHA was synthesized by Assistant Professor Dr. Chamnan Patarapanich et al., (1996). The structure of VHA consists 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. Thus, with reference to VPA this study was aimed to determined :

1. Anticonvulsant efficacy of VHA
2. Acute toxicity and neurotoxicity of VHA
3. Degradation of VHA by liver and brain homogenates .