Chapter I

Introduction

Epilepsy

The word epilepsy comes from the Greek "epilambanein", which means to seizure or to attack. This initially referred to an affliction by any diseases, but over time became specific to epileptic seizures. Sometimes the words seizure disorder, convulsion and epilepsy are often used interchangeably, although they do not really mean the same thing.

Convulsions are involuntary spasmodic contractions of the muscle. Most people probably think that a convulsion is a generalized tonic-clonic reaction that occurs with grand mal or major motor epilepsy, although it occurs in other situations as well (Collins, 1983).

A seizure caused by abnormal and excessive discharges of a set of neurons in the brain which may result from epilepsy or other disorders. The clinical manifestation consists of sudden and transitory abnormal phenomena which may include alterations of conscious, motor, sensory, autonomic or psychic events. This is a finite event with a beginning and an end (Rall and Schleifer, 1990; Roger and Porter, 1991; Sander and Shorvon, 1996).

The term epilepsy refers to a syndrome of episodic brain dysfunction which characterized by recurrent (two or more) epileptic seizures, unprovoked by any immediate identified causes (Sander and Shorvon, 1996).

Epidemiologic Aspects of Epilepsy

Epilepsy is one of the most common neurological disorders which affects about 1% of global population. The incidence is highest in the first 10 years of life and elderly. The prevalence of epilepsy among boys and men is somewhat higher than among girls and women , but there is no age specific trend. Also in blackmen the prevalence is two

times more common than whitemen. Besides, the prevalence of epilepy is greater in areas of the world that have higher incidence of brain injury due to high rates of infections, poor perinatal care and frequent head trauma (Rogawski and Porter, 1990; Smith and Buelow, 1996; Sander and Shorvon, 1996).

Etiologic Aspects of Epilepsy

Epileptic seizures may be a natural reaction to physiologic stress or transient systemic injury (reactive seizures) or they may indicate an epileptic disorder. This disorder may reflex intrinsic, non progressive, and presumably hereditary cerebral disturbances, with seizures as the only manifestation of abnormal brain function without a known environmental cause (primary or idiopathic epilepsy), or may be a symptom of some known pathologic processes affecting the brain (secondary epilepsy). Several factors often exist in the same patient, and commonly the combination of a cerebral insult and a genetic predisposition determines the appearance of epileptic seizures (Engel, 1992).

Genetic Factors

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 may contribute to the development of epilepsy in three ways : 1) an individual may inherit a low threshold for seizures; 2) genetic traits underline certain specific primary epileptic conditions; and 3) many inherited diseases of the brain are associated with structural disturbances that produce seizures (Engel, 1992).

Primary epilepsies account for 30% of chronic epileptic disorder. Most of the patient have a family history of epilepsy. More than 150 autosomal inherited disorders are associated with epilepsy, for example, trisomy 13, 18, 21, and 22. However, not all patterns like this case have seizures (Engel, 1992; Smith and Buelow, 1996).

In addition, the susceptibility of individual brains to development of generalized convulsions can occur as a reaction to insults such as sleep deprivation, alcohol, fever and head trauma etc. Recurrent generalized convulsions may also be induced by reversible infectious, toxic, or metabolic process and are limited to the period of systemic illness. Occurrence of such reactive seizures generally indicates an inherited low threshold for seizures. The most commonly encountered reactive seizures are the benign febrile convulsions. Individuals with low convulsive thresholds are also more likely to develop chronic recurrent seizures of all types if irreversible brain injury occurs for other reasons (Engel, 1992).

Acquired Factors.

Although there are many known acquired causes of epilepsy, for most patients the cause of a chronic seizure disorder remains unknown. The specific cause of epilepsy was found in only 23% of the patient. Of the known causes, the most common are trauma, vascular disorder, neoplasia, congenital abnormality, infection and birth anoxia (Smith and Buelow, 1996).

Nature and Mechanism of Seizure.

It would be a gross oversimplification to suggest a single mechanism or cause of epilepsy. Because a common denominator has so far not been found and a variety of genetic, environmental, and even normal physiologic factors are important contributing factors to the appearance of seizures.

For an epileptic seizure to occur, two basic phenomena must coexist : there must be excessive neuronal excitability (hyperexcitation) and abnormal neuronal synchronization (Smith and Buelow, 1996).

In cellular electrophysiological studies, with various experimental models of epilepsy that produce neuronal hyperexcitability, a great deal has been discovered about the hyperexcitable short-circuiting neuron. It is called the depolarization shift (DS) which consists of a large depolarization of the neuronal membrane associated with a burst of action potentials. The summation of these action potentials produces an

" interictal spike" (a sharp wave form) of EEG that can be recorded from the surface of the brain or from the skull (Figure 1.).

Although many factors are proposed to be the cause of interictal seizures which leads to epileptogenesis. However it remains the controversial, that whether the interictal spike triggers a seizure, inhibits a seizure, or is an epiphenomenon with respect to a seizure occurrence in an epileptic brain (Smith and Buelow, 1996; McNamara,1996; Heineman and Jones, 1990).

For hyperexcitation : Neuronal hyperexcitability can be due to metabolic dearrangements of the microenvironment of the neuron that leads to increases in extracellular potassium. In normal neuronal function, glutamate plays an important role in excitation which leads to sodium and then calcium entrance into the neuron. The excessive neuronal excitability caused by enhanced glutamate level leads to excitotoxic which causes loss of GABAergic neurons resulting in elimination of GABA-mediated inhibition and subsequently leading to pathologic hyperexcitability (Smith and Buelow, 1996).

Although there are many different ways to produce hyperexcitability in neurons, analyses of multiple *in vitro* models confirmed the importance of synaptic function in initiation of a seizure. The reduction of inhibitory synaptic function could lead to epileptic form activity and that activation of excitatory synapses could be pivotal in initiation of a seizure. These include the important factor, voltage-regulated ion channels as well (McNamara, 1996).

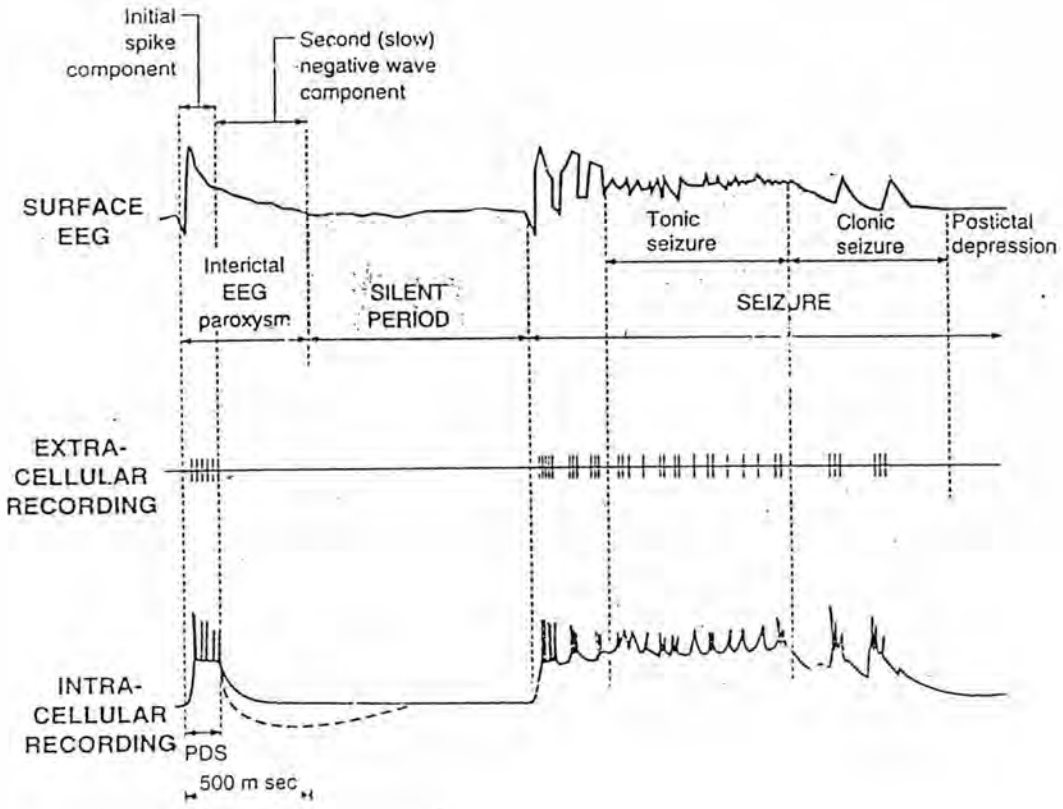

Figure 1. Relations among cortical EEG, extracellular, and intracellular recordings in a seizure focus induced by local application of a convulsant agent to mammalian cortex (Adapted from Ayala et al., 1973).

Classification of Seizures

The classification of epilepsy is complex. Because epilepsy can arise from a variety of underlying conditions and pathophysiologic mechanisms, besides most cases are classified as idiopathic or cryptogenic. Accurate classification of seizure is essential for understanding epileptic phenomena, developing a rational plan of investigation and as a guideline for drug treatment (Smith and Buelow, 1996).

The most widely accepted classification of seizures which used today is the classification of epileptic seizures developed by the International League Against Epilepsy (ILAE) in 1981 (Table 1.). This system classifies seizures by clinical symptoms supplement by EEG data (attack and pattern of EEG) (Pedley, Scheuer and Walczak, 1995).

Inherent in the classification are two important physiologic principles. First, seizures are fundamentally of two types. One with onset limited to a part of one cerebral hemisphere (partial or focal seizures) and the other involving both hemispheres widely from the outside (generalized seizures). Second, seizures are dynamic and evolving ; clinical expression is determined as much by the sequence of spread of electrical discharge within the brain as by the area where the ictal discharge originates (Pedley, Scheuer and Walczak, 1995). More detail was shown in Table 1.

The type of epileptic seizure determines the drug selected for therapy. For primary generalized tonic-clonic seizures, phenytoin or valproate is the drug of choice; carbamazepine and phenobarbital are second-line drugs. The drugs of choice for the various seizures are listed in Table 2. (Smith and Buelow, 1996).

Table 1. Internation Classification of Epileptic Seizures

I. Partial seizures
    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 comsciousness
            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 Leagre Against Epilepsy

Seizure Type	Anticonvulsant	Adjunct
Simple partial seizures	CBZ, PHT, VPA	CLN, FEL, GAB, LAM, MET, PB, PRM, TIA, TOP, VGB
Complex partial seizures	CBZ, PHT, VPA	CLN, FEL, GAB, LAM, MET, PB, PRM, TIA, TOP, VGB
Partial seizures with secondary generalization	CBZ, PHT, VPA	CLN, FEL, GAB, LAM, MET, PB, PRM, TIA, TOP, VGB
Primary generalized convulsions	VPA, PHT	CBZ, CLN, FEL, GAB, LAM, TIA, TOP, VGB
Absence seizures	ETX, VPA	LAM, MET
Myoclonic seizures	VPA	CLN, LAM
Infantile spasms	ACTH, CLN, VPA	
Lennox-Gastaut	CLN, VPA	ACTH, FEL, LAM, VGB

ACTH = Adrenocorticotropic hormone or prednisone; CBZ = carbamazepine; CLN = clonazepam (or other benzodiazepines); ETX = ethosuximide; FEL = felbamate; GAB = gabapentin; LAM = lamotrigine; MET = methsuximide; PB = phenobarbital; PHT = phenytoin; PRM = primidone; TIA = tiagabine; TOP = topiramate; VGB = vigabatrin; VPA = valproic acid or divalproex.

Table 2. Drug treatment in epileptic seizures.

Amino Acid Neurotransmitter Alteration within Epileptic Brain

Amino acid neurotransmitters in the mammalian CNS have been separated into two general classes: excitatory amino acids, glutamate and aspartate, which exert an excitatory action by depolarizing neurons; and inhibitory amino acids, GABA and, to a lesser extent, glycine, which exert an inhibitory action by hyperpolarizing neurons.

Inhibitory Amino Acids

γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain while glycine is an inhibitory neurotransmitter mainly in the spinal cord, acting on its own receptor, which functionally resembles the $GABA_A$ receptor (Trevor and Way, 1995).

GABA exerts an inhibitory action in all forebrain structures, and 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 inhibition. In the former, GABA acts on a presynaptic terminal of an excitatory neuron to prevent release of transmitter; this form of inhibition is found predominately in the spinal cord. Postsynaptic inhibition is the main inhibitory mechanism found in the brain and it is at this site that most of epileptic drug exert their action (Davies and Richens, 1993).

Impairment of GABA functions produces seizures, whereas enhancement results in an anticonvulsant effect. Numerous steps in GABA synaptic function are relevant to epileptogenesis : a) GABA synthesis; b) GABA release; c) GABA transport; and d) activation of receptors, subtypes A and B (Olsen and Avoli , 1997).

Basic Physiology of GABA

In mammals, GABA is found in high concentrations (μmoles/g) in the brain and spinal cord (Cooper, Bloom and Roth,1991).

Glucose, the main energy source of the brain, is the principle precursor for GABA production *in vivo*. The first step in GABA shunt (central to the GABA system) is the transmination of α-ketoglutarate, formed from glucose metabolism in the Krebs cycle, by GABA α-oxaloglutarate transaminase (GABA-T) into glutamic acid.

GABA was synthesized from glutamic acid by the enzyme glutamic acid decarboxylase (L-glutamate decarboxylase, GAD). GAD expressed only in cells that used GABA as a neurotransmitter. So GAD was an excellent marker for GABAergic neurons in the CNS. GABA is metabolized by GABA-T to form succinic semialdehyde (SSA) and then to succinic acid by succinic semialdehyde dehydrogenase (SSADH) and can then reenter the Krebs cycle (Figure 2.) (McGeer, Eccles and McGeer, 1987: Delorey and Olsen, 1994).

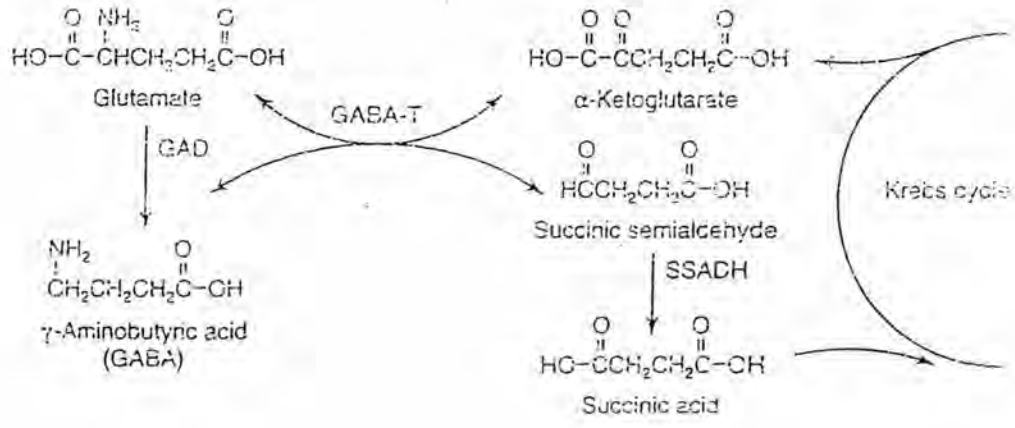

Figure 2. The scheme of GABA synthesis.

After GABA was released from nerve endings, the action of GABA at the synapse is terminated by being reuptaked into presynaptic nerve terminals or picked up by membrane transport of glia cells (McGeer, Eccles and McGeer, 1987; Cooper, Bloom and Roth, 1991; Delorey and Olsen, 1994).

GABA Receptors

GABA release into the synaptic cleft is stimulated by depolarization of presynaptic neuron and exerts its inhibitory action on postsynaptic membrane by acting at specific receptor sites to activate a chloride (Cl^-) channel.

The actions of GABA are mediated by at least two distinct classes of receptors, termed $GABA_A$ and $GABA_B$. They differ in their pharmacological, electrophysiological and biochemical properties (Delorey and Olsen, 1994). At the postsynaptic level, GABA mediated both rapid inhibition through $GABA_A$ receptor (Figure 3), which is a member of the ligand-gated ion channel superfamily, and slow inhibition through $GABA_B$ receptor, which is a member of G-protein-linked receptor superfamily. In both cases, GABA acts by increasing membrane conductance for an ion having an equilibrium potential near or more negative than resting membrane potential; in this way the neuron hyperpolarizes, thus preventing cell firing. The difference is that $GABA_A$ receptors act by generating a large Cl^- conductance, where as $GABA_B$ receptors increase potassium (K^+) conductance.

Overall, the effects of GABA are depressant. Therefore, the major role of GABA in the epileptic brain is that of excerting an "antiepileptic influence" (Olsen and Avoli , 1997).

Excitatory Amino Acids

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 a fast predominant excitatory neurotransmitter in CNS, besides being the essential immediate precursor for the synthesis of GABA and important intermediate in neuronal metabolism (Greenamyre and Porter, 1994). Enhanced responsiveness to activation on N-methyl-D-aspartate (NMDA) receptor, a major receptor type of glutamate, may be involved in various acquired forms of epilepsy. These suggested that either enhanced glutamate release or impaired uptake contibutes to seizure initiation (Loscher, 1993).

Basic Physiology of Glutamate

Glutamate is formed mainly from the Krebs cycle intermediate, α-oxoglutarate, by the action of GABA-aminotransferase (Travor and Way, 1995). The neurotransmitter pool of glutamate is stored in synaptic vesicles and, upon depolarization, is released into the synaptic cleft in a calcium (Ca^{2+}) dependent fashion. It exerts excitatory action by interaction with postsynaptic ionotropic receptors resulting in opening of cation channels and subsequently membrane depolarization.

The action of glutamate is terminated by high-affinity, Na^{+}-dependent uptake carriers located in both neurons and glia (Greenamyre, and Porter, 1994).

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 classified as NMDA, receptor that produces a much slower response, and non-NMDA , AMPA and KA, which insensitive to the synthetic agonist NMDA (Lipton and Rosenberge, 1994).

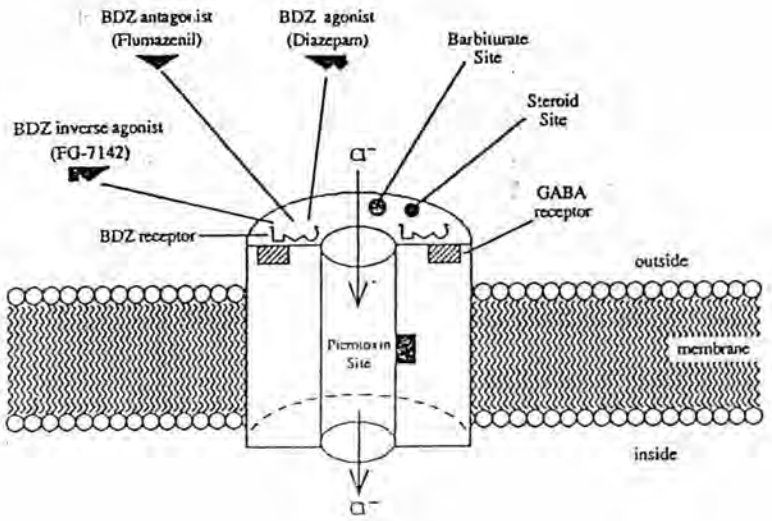

Figure 3. Schematic illustration of the $GABA_A$ receptor complex.

The Mechanism of Action of Antiepileptic Drug

Therapy of epilepsy has three goals : 1) to eliminate seizures or reduce their frequency to the maximum extent possible; 2) to avoid the side effects associated with long-term treatment; 3) to assist the patient in maintaining or restoring normal psychosocial and vocational adjustment (Pedley, Scheuer and Walczak, 1995).

There are two general ways in which drug might abolish or attenuate seizures : through effects on pathologically altered neurons of seizure foci to prevent or reduce their excessive discharge and through effects that would reduce the spread of excitation from seizure foci. Most, if not all, antiepileptic agents that are presently available act at least in part by the second mechanism, since all modify the ability of the brain in response to various seizure-evoking stimuli (Rall and Schleifer, 1990).

So the target mechanism of antiepileptic drugs can be broadly classified into one or more of three mechanistic categories; 1) enhancement of GABAergic transmission; 2) reduction of excitatory (particularly glutamatergic) transmission, or 3) modulation of ionic conductances (Na^+, Ca^{2+} or K^+) (Trevor and Way, 1995; Upton, 1994).

Mechanisms of Established Antiepileptic Drugs

Phenobarbital was the first synthetic anticonvulsant discovered in 1912. At present the drug development for antiseizure usually begins with screening experimental models. MES, PTZ and electric kindle model, are the most commonly employed in the search for effective antiepileptic drugs (AEDs) (McNamara, 1996; White, 1997). In general AEDs were thought to exert their anticonvulsant activity by three major mechanism (White, 1997).

1) Phenytoin (PHT) and carbamazepine (CBZ) are effective against all types of generalized tonic-clonic and partial seizures but not absence seizure (Trevor and Way, 1995). These AEDs reduce the frequency of sustained repetitive firing of action potentials in culture by stabilizing the inactive forms of the Na^+ channels and delay its rate of recovery from inactivation. Although PHT and CBZ have qualitatively

similar actions on Na^+ channels, the actions are quantitatively somewhat different. PHT has a stronger slowing effect than CBZ (McNamara, 1996; Meldrum, 1996; White, 1997).

2) Ethosuximide (ESM) and trimethadone (TMD) are relatively effective in preventing seizures induced by pentylenetetrazol (PTZ) which are primarily useful for the treatment of absence seizures. Blocking of low-threshold T-type Ca^{2+} current was believed to account for their anticonvulsant effect (Mcdonald and Kelly, 1993; White, 1997).

3) Benzodiazepines (BZD) and barbiturates (BBT) limit high-frequency repetitive firing of action potentials only at high concentrations. Their anticonvulsant activity is believed to be related principally to their capacity to enhance inhibitory neurotransmission by modulating the $GABA_A$ receptor complex (White, 1997).

BZD and BBT interact with different α and β subunit triggering Cl^- channels opening. BBT acts mainly by increasing the mean channel opening duration while BZD increases opening frequency but does not directly initiate Cl^- current (Trevor and Way, 1995). $GABA_A$ receptors composed of α_1 and β_1 subunits are sensitive to BBT but not BZD, while the transient co-expression of the γ_2, α_1 and β_1 subunits results in sensitivity to both BZD and BBT (White, 1997).

Most of these established AEDs have a limited spectrum of antiepileptic activity, and all have certain negative properties that limit utility and patient management. At least 25% of epilepsy patients remain refractory to these AEDs. Because the drugs used currently not only fail to control seizure activity in some patients, but they frequently cause side effects that range in severity from minimal impairment of the CNS to death from aplastic anemia or hepatic failure (McNamara, 1996). So the requirement for new AEDs with improved clinical profiles is quite clear.

Mechanisms of Action of New Antiepileptic Drugs

Increasingly, AEDs development has produced novel molecules that enhance inhibitory neurotransmission by acting on GABA transport, metabolism and receptor activity or by reducing excitatory neurotransmission mediated primarily by glutamate and other excitatory amino acids.

1) Vigabatrin (VGB)

VGB, a close chemical analogue of GABA is a catalytic or irreversible inhibitor of GABA-T . 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 (Meldrum, 1996; White, 1997).

2) Gabapentin (GBP)

GBP, a cyclohexane derivative of GABA, expected to enhanced GABA mediated inhibition but it did not act on $GABA_A$ or $GABA_B$ receptors (McNamara, 1996; White, 1997).

GBP blocks MES-induced convulsions in experimental animals and to a lesser extent, PTZ-induced convulsions. Therefore GBP is expected to be effective in the treatment of human partial and generalized tonic clonic seizures but not absence seizures (Mcdonal and Kelly, 1995; Smith and Buelow, 1996). Although, the mechanism of action of GBP is not established, the current hypothesis suggests that GBP alters brain amino acids, possibly both the excitatory and inhibitory amino acids. Its possible mechanism might be an increase in GABA synthesis (Loscher et al, 1991; Kalviainen, Keranen, Rickkinen, 1993; Macdonal and Kelly, 1995; McNamara, 1996) and antagonism on NMDA receptors (Kalviainen, Keranen, Rickkinen, 1993; Mcdonal and Kelly, 1995).

3) Tiagabine (TGB)

In animals, TGB is active against several types of chemically induced seizures, however it is active in MES test only at high doses.

The action of TGB on GABA uptake leads to increased synaptic concentrations of GABA and consequent enhancement and prolongation of GABA-mediated inhibitory neurotransmission, which are assumed to be the basis of TGB's anticonvulsant activity (White, 1997).

4) Lamotrigine (LTG)

Lamotrigine is active against MES-induced tonic seizures but is ineffective against scPTZ-induced clonus (Kalviainen, Keranen, Rickkinen, 1993; McNamara, 1996; White, 1997) suggesting that LTG should be effective against generalized tonic seizures and other childhood epilepsy syndrome (Smith and Buelow, 1996). Furthermore, it was found that LTG blocked sustained repetitive firing in cultured mouse spinal cord neurons and inhibited the release of glutamate and aspartate evoked by the sodium channel activator, veratrine. Therefore, it is likely that LTG decreases presynaptic release of excitatory amino acid, principally glutamate, by its action at the voltage sensitive Na^+ channel (Rogawski and Porter, 1990; Wilder, 1994; Macdonal and Kelly, 1995; White, 1997).

5) Felbamate (FBM)

In animal models, FBM is effective against MES-induced tonic seizure as well as against certain chemically induced clonic seizures. It is also effective in kindling models. Thus, it is suggestive that FBM should be effective in partial seizures (White, 1997).

The mechanism of action of FBM is not certain. It was believed to possess multiple mechanisms of action. FBM inhibited the NMDA evoked response by modulating glutamate receptor function through an action on glycine, a co-agonist of glutamate (Upton, 1994; White, 1997). In addition FBM also enhances GABA evoked chloride current *in vivo*.

Although, FBM remains a mechanistically very interesting AEDs with an apparently broad anticonvulsant profile, its clinical utility has unfortunately been markedly limited by the serious hematologic and hepatic toxicity (Smith and Buelow, 1996; White, 1997).

6) Topiramate (TPM)

TPM is highly effective against MES but it is relatively weak against scPTZ-induced seizure. Several lines of evidence suggest that TPM has multiple mechanisms of action contributing to its broad anticonvulsant profile. The effects that TPM reduces sustained repetitive firing, spontaneous burst firing, and Na^+ currents might be attributable to a state-dependent block of voltage-dependent Na^+ channels. TPM exerted effects on non-NMDA glutamate receptors by reducing kainate-evoked inward currents. Furthermore, TPM was found to be able to potentiate the effect of GABA by interaction at $GABA_A$ subunit (Macdonal, 1995; White, 1997).

One or more of these findings may account for the efficacy of TPM in partial secondarily generalized and absence seizures (Meldrum, 1996; White, 1997).

Valproic acid

Valproic acid , was first synthesized in 1882 by Burton and being used as a solvent in the search for drugs effective against seizure. No therapeutic application of VPA was known until the discovery of its anticonvulsant properties in 1963, by P. Eymard. The product was first marketed as "Depakine" in France in 1967 (Chapman, Meldrum and Simand, 1982).

Comparative analysis of anticonvulsant potency and safety margin, utilizing the classical animal model for anticonvulsant screening, show that VPA is less potent than the other three established antiepileptic drugs : phenobarbital, phenytoin and carbamazepine. So that there is substantial need to develop improved derivatives of VPA (Bialer, Haj-Yehia, Badir and Hadad, 1994; Penry and Dean, 1989).

Chemistry

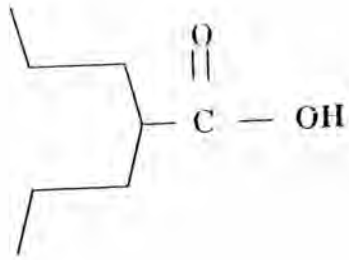

Figure 4. The chemical structure of Valproic acid (di-n-propylpentanoic acid).

VPA, a colourless acid, is an anticonvulsant that differs markedly from all other anticonvulsant drugs in clinical use, It has a simple aliphatic molecular structure, branched chain fatty acid, which 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 (Porter, Brian and Meldrum, 1994).

Pharmacological Effects.

Valproate is very effective against absence seizures. Although ethosuximide is the drug of choice when absence seizures occur alone, VPA is preferable if the patient has concomitant generalized tonic-clonic attacks (Porter, Brian and Meldrum, 1994). VPA is more effective in generalized than in partial epilepsies and it is unique in its ability to control certain types of myoclonic seizures (Lewis, 1978).

Mechanism of Action.

VPA is a unique anticonvulsant with a broad spectrum of activity against several types of seizures, particularly absence seizures. It has repeatedly been suggested by experimental and clinical data that VPA acts through a combination of several mechanisms (Loscher, 1993).

1) Effects on the GABA system.

GABA, is one of the principal inhibitory neurotransmitter in CNS. It is generally accepted that impairment of GABAergic inhibitory neurotransmission can lead to convulsion, whereas potentiation of GABAergic transmission results in anticonvulsant effects (Loscher, 1993).

The finding that VPA increased brain GABA levels could be resulted from an activation of GABA synthesis enzyme, GAD (Devis, Peter and McTavish, 1994), and/or inhibition of GABA-T, which catalyses the degradation of GABA to succinic semialdehyde (SSA) (Browne, 1980; Johnston and Slater, 1982; Loscher, 1993).

2) Effects on membrane ion channels.

Valproate exerts its action by increasing extracellular K^+ concentrations or blockade of Na^+ channels resulting in a reduction of high frequency repetitive firing of neurons (Johnston and Slater, 1982; Fromm, 1992; Davis, Perer and McTavish, 1994). Although it is as effective as ethosuximide in blocking absence seizures, the effect of VPA on T-type Ca^{2+} channels was not clear (Mcdonald and Kelly, 1995).

3) Effects on amino acid neuro transmitters other than GABA.

Valproic acid has been reported to reduce neurotransmission mediated by excitatory amino acids such as aspartic acid, glutamic acid and gamma-hydroxybutyric acid (GHB) (Davis, Peter and McTacish, 1994). Since GHB produces absence-like epileptic seizures in animals, reduction of GHB release could be an important factor in the anti-absence action of VPA (Loscher, 1993).

Postsynaptic action of VPA on the response to GABA, glycine and glutamate has been studied by iontophoretic technique. Application of VPA, selectively augments postsynaptic GABA-mediated inhibition. However, the effect of VPA on glycine and glutamate responses was negligible (Johnston and Slater, 1982).

Pharmacokinetic Properties.

Valproic acid is rapidly and completely absorbed after oral administration. Peak plasma concentrations of VPA are reached within 1 to 4 hours after admistration as capsules and 15 minutes to 2 hours after admistration as syrup. Absorption is delayed if VPA is taken after meal or in enteric-coated tablets.

VPA has pKa of 4.7. The absolute bioavailability of VPA is 68-100 percent when it is given as tablets and 86-100 percent when it is given as a solution (McNamara,1996; Browne, 1980).

The distribution of VPA is primarily limited to the blood and extracellular fluid (Chapman, Keane, Meldrum and Simiand, 1982). The apparent volume of distribution for VPA is about 0.2 liter/kg. Its extent of binding to plasma proteins is usually about 90% (McNamara, 1996). VPA crosses the placental barrier and is also excreted in breast milk (Lewis, 1978). The cerebrospinal fluid in man contains VPA levels similar to the free blood levels. Levels in brain tissue varie between 6.8 and 27.9% of plasma levels. Therapeutic levels of VPA range from 50-100 μg/ml (Porter, Brian and Meldrum, 1994).

VPA is metabolized rapidly, almost entirely by the liver. At least five main metabolic pathways for VPA have been described in humans : glucuronidation, β-oxidation, ω, ω_1, and ω_2 oxidation. Its major metabollic pathway appears to be β-oxidation (Browne, 1980). Only 1 to 3% of an administered dose of VPA is excreted unchanged in the urine, while most of both the parent drug and several of its metabolites undergo conjugation with glucuronic acid. 2-En-valproic acid, a β-oxidation derivative, probably contributes to the anticonvulsant activity of VPA, while another metabolite, 4-En-valproic acid, may be involved in both hepatotoxicity and embryo toxicity. The half-life of VPA is approximately 9-17 hours which varies with age and is reduced in patient receiving more than one drug (Lewis, 1978; Davis, Peters and McTavish, 1994).

Therapeutic Uses.

Valproic acid is marketed in 250 mg capsule and syrup containing the equivalent of 250 mg valproic acid per 5 ml, as the sodium salt. The initial daily dose usually is 15 mg/kg BW., and this is increased at weekly interval by 5 to 10 mg/kg BW. per day to maximum daily dose of 60 mg/kg BW. However, the dose requirement of VPA should be adjusted, depending on patient's response, liver disease, renal impairment, adverse reactions and combination with other drugs (Lewis, 1978; Browne, 1980; Davis, Peters and McTavis, 1994; McNamara, 1996).

Drug Interactions

Concomitant administration of VPA affects the plasma concentrations of other drugs by displacement from plasma protein/or inhibition of hepatic metabolism (Davis, Peters and McTavish, 1994).

Protein binding of VPA (90%, mainly albumin) results in a longer retention of the drug, and its temporary inactivation. Furthermore, in combination with other drugs, VPA inhibits the metabolism of several drugs, including phenobarbital, phenytoin and carbamazepine, leading to higher steady state concentrations of these agents (Porter, Brian and Meldrum, 1994).

Hepatic enzyme-inducing drugs such as phenytoin, carbarmazepine and phenobarbital can significantly reduce steady-state plasma valproic acid concentrations, resulting in suboptimal seizure control (Davis, Peters and McTavish, 1994; Ferrendelli, 1995).

Results in animal studies suggest that valproic acid and alcohol may naturally inhibit each other's elimination rates, resulting in potentiation of their respective CNS depressant effect (Lewis, 1978).

Toxicity

Almost all antiepileptic drugs potentially produce undesirable side effects (Engel, 1992). The most common adverse effects associated with valproic acid are mild to moderate in severity with gastrointestinal

disturbances (nausea, vomiting, dyspepsia), weight gain, neurological effects (tremor, fatigue, somnolence, dizziness and headache) being the most frequently reported (Davis, Peters and McTavish, 1994). These problems can be solved by reducing the dosage (Rall and Schleifer, 1990).

The idiosyncratic reactions (serious adverse reactions) such as thrombocytopenia, hepatic failure, aplastic anemia and teratotoxicity have been reported with valproate therapy. The serious ones are hepatotoxicity and teratogenicity.

VPA-induced fatal hepatotoxicity occurs more frequently in patients of less than two years olds receiving polytherapy and generally manifests within 6 months of therapy initiation (Davis, Peters and McTavish, 1994).

In animal studies, dose related teratogenesis with VPA usually manifested as retarded fetal growth and major developmental abnormalities, including skeletal defects (Lewis, 1980). The risk of neural tube defects, predominantly spina bifida aperta, estimated in infants born to women treated with VPA during pregnancy about 1-2 % (Davis, Peters and McTavish, 1994).

Amide 1C

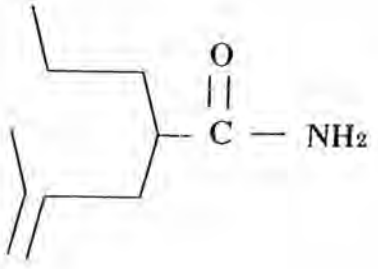

Figure 5. The chemical structure of Amide 1C.

Amide 1C is a newly synthesized VPA analog which was synthesized by Assistant Professor Dr. Chamnan Patarapanich and Mr. Pornchai Rodesittisuk. The structure of Amide 1C consists of two parts, one is the branched chain with double bond of 4-methyl-2-propyl-

4-pentanoic acid and the other is $CONH_2$ group of valpromide. Both of them have potent anticonvulsant efficacy, higher protective indices and safety ratio (TD_3/ED_{97}) than valproic acid (Bialer,Haj-yehia, badir and Hadad,1993-1994). The chemical structure of Amide 1C was designed in expectation for a VPA analogue with a higher potency and less toxicity than VPA. Thus, this study was aimed to determine :

1. Anticonvulsant efficacy of Amide 1C
2. Acute toxicity and neurotoxicity of Amide 1C
3. Effects of Amide 1C on the level of excitatory and inhibitory amino acid neurotransmitters in the cerebral cortex of anesthetized rats by microdialysis technique.