Chapter I

Introduction

1. Definition and Epidemiology

Epilepsy was known to the ancient Babylonians and was described by Hippocrates, who considered it as disease of the brain. In 1890, John Hughlings Jackson concisely defined epilepsy as "...an occasional excessive and disordered discharge of nerve tissue." Epilepsy is not a disease but a syndrome of many different cerebral disorders characterized by recurrent seizures, the clinical manifestration of an abnormal discharge of a set of neurons in the brain, due to excessive fluctuations in cerebral electrochemical balance (Hauser, Annegers, and Anderson, 1983; Fukuzako and Izumi, 1991). More recently, epilepsy has been defined as recurrent convulsive or non-convulsive seizures caused by partial or generalized epileptogenic discharges in the cerebrum (Menkes, 1990).

Epilepsy afflicts at least 1-2 million people in the United States and about 20-40 million people worldwide (Rall and Schleifer, 1990). It is more common in the children than in adults, with a prevalence of 5.2-8.1 per 1,000 children below the age of 10 years (Menkes, 1990). According to a considerable number of epidemiological studies from many countries its prevalence and incidence range from 0.15-1.95 % and from 0.02-0.05 %, respectively. Males tend to predominate in both prevalence and incidence (Fukuzako and Izumi, 1991).

2. Classification

The classification of epilepsy is complex, and can be based on the etiology, pathology, age of onset, clinical seizure, electroencephalogram (EEG) findings, and prognosis (Fukuzako and Izumi, 1991). The etiologic category is generally subdivided by the presence or absence of an antecedent neurologic insult. From the mid-19th century, epilepsies have been classified into two major groups. Patients with epilepsy in the absence of any history of prior neurologic insult are usually categorized as "primary or idiopathic epilepsy." If historical information identifies a reasonable predisposing factor, the epilepsy is termed "secondary or symptomatic epilepsy." (Hauser, Annegers, and Anderson, 1983; Menkes, 1990; Rall and Schleifer, 1990; Gastaut and Zifkin, 1992)

A revised classification of individual seizure types was accepted in 1981 by the General Assembly of the International League Against Epilepsy (ILAE) and has been widely used in the management of epilepsy (Commission on Classification and Terminology of the ILAE, 1981). However, this dose not accurately reflect the complicated epileptic syndrome which is characterized by a cluster of signs and symptoms customarily occuring together. These include such items as type of seizure, etiology, anatomy, precipitating factors, age of onset, chronicity, diurnal and circadian cycling, and sometimes prognosis. The classification of epilepsies and epileptic syndromes was therefore revised in 1985. Recently, a new classification of epilepsies and epileptic syndromes has been published by the Commission on Classification and Terminology of the ILAE (1989). This classification is not totally satisfactory and is still undergoing evaluation, although several of the epileptic syndromes are quite well defined. (Fukuzako and Izumi, 1991)

The 1981 classification of epileptic seizures is considered pragmatic for clinical use , and serves as a useful tool for guiding decisions about when to

treat epilepsy and how to choose among the antiepileptic drugs (Rall and Schleifer, 1990; Fukuzako and Izumi, 1991). For propose of drug treatment, it is more useful to classify patients according to the type of seizure they experience. A simplified form of the proposal from the Commission on Classification and Terminology of ILAE (1981), based on the clinical manifestrations of the attacks and the pattern of the EEG, is presented in term of seizure types and characteristics in Table 1 (Rall and Schleifer, 1990).

3. Etiology

Recurrent seizures are thought to result from a genetic predisposition, underlying neuropathologic changes, and chemicophysiologic alterations in the nerve cell and its connections (Menkes, 1990).

Epilepsy has been considered a genetic disease for centuries. As early as 450 B.C. in his treatise, *The Sacred Disease*, Hippocrates concluded that epilepsy was inherited (Hauser, Annegers, and Anderson, 1983). Numerous studies suggest that the genetic susceptibility to seizure is normally distributed in the general population and that there is a threshold above with the condition becomes clinically evident (Menkes, 1990). Genetic factors appear to be most significant in patients with various primary epilepsies (Menkes, 1990; Hopkins, 1993).

Seizures can occur in patients with almost any pathologic process that affect the brain, such as perinatal injury, trauma, tumours, stroke, vascular disease, and degenerative disorders (Menkes, 1990; Hopkins, 1993). Several areas of the brain, especially the hippocampus, appear to be particularly vulnerable to the homeostatic alterations produceed by recurrent and prolonged seizures. Cellular changes are also seen in the cerebellum and to a lesser extent in the cerebral hemispheres. (Menkes, 1990)

Table 1. Classification of Epileptic Seizures * (From Rall and Schleifer, 1990)

SEIZURE TYPE †		CHARACTERISTICS
I. *Partial Seizures* (Focal, Local Seizures)	A. Simple partial seizures	Various manifestations, without impairment of consciousness, including convulsions confined to a single limb or muscle group (*Jacksonian motor epilepsy*), specific and localized sensory disturbances (*Jacksonian sensory epilepsy*), and other limited signs and symptoms depending upon the particular cortical area producing the abnormal discharge
	B. Complex partial seizures	Attacks of confused behavior, with impairment of consciousness, with a wide variety of clinical manifestations, associated with bizarre generalized EEG activity during the seizure but with evidence of anterior temporal lobe focal abnormalities even in the interseizure period in many cases
	C. Partial seizures secondarily generalized	
II. *Generalized Seizures* (Convulsive or Nonconvulsive)	A.1. Absence seizures	Brief and abrupt loss of consciousness associated with high-voltage, bilaterally synchronous, 3-per-second spike-and-wave pattern in the EEG, usually with some symmetrical clonic motor activity varying from eyelid blinking to jerking of the entire body, sometimes with no motor activity
	A.2. Atypical absence seizures	Attacks with slower onset and cessation than is usual for absence seizures, associated with a more heterogeneous EEG
	B. Myoclonic seizures	Isolated clonic jerks associated with brief bursts of multiple spikes in the EEG
	C. Clonic seizures	Rhythmic clonic contractions of all muscles, loss of consciousness, and marked autonomic manifestations
	D. Tonic seizures	Opisthotonus, loss of consciousness, and marked autonomic manifestations
	E. Tonic-clonic (*grand mal*) seizures	Major convulsions, usually a sequence of maximal tonic spasm of all body musculature followed by synchronous clonic jerking and a prolonged depression of all central functions
	F. Atonic seizures	Loss of postural tone, with sagging of the head or falling

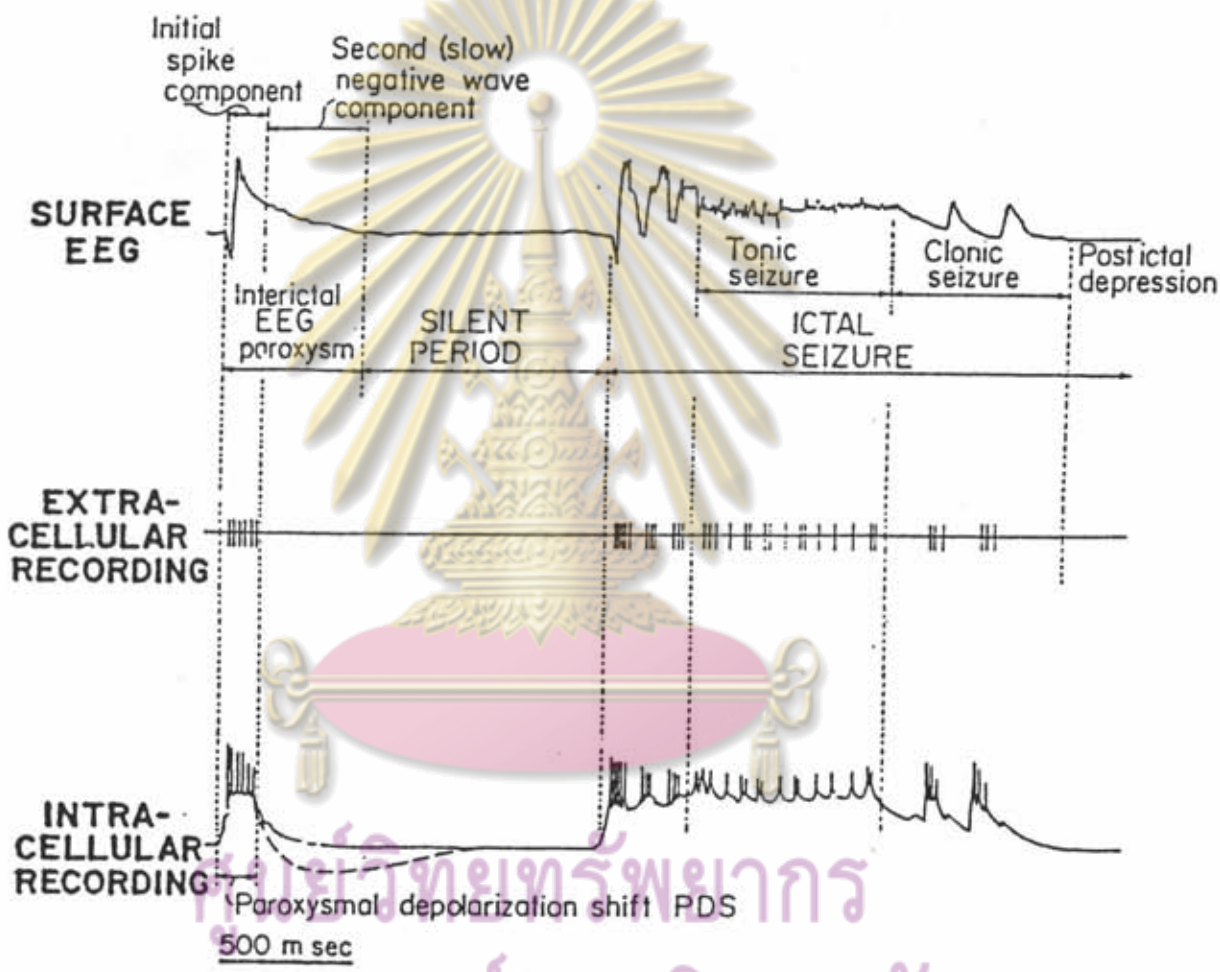
* Modified from the proposal from the Commission on Classification and Terminology of the International League Against Epilepsy (1981).

† Additional seizure types are presently unclassified owing to incomplete data.

From a neurophysiologic point of view, an epileptic seizure has been defined as an alteration of central nervous system (CNS) function resulting from spontaneous electrical discharge in a disease population of cortical gray matter or the brainstem (Menkes, 1990). Although a major seizure may involve nearly the entire CNS, in its simplest form a focal seizure represents an abnormality of function in only a small portion of the brain, the epileptic focus that John Hughlings Jackson established this basic concept many years ago (Ayala et al., 1973). Epileptogenesis requires a set of epileptic neurons and circuitry to permit multicellular synchronization (Menkes, 1990).

The transmembrane potentials of many neurons within an epileptic focus undergo a sudden, long-lasting depolarization coincident with the cortical paroxysm. This was named the "paroxysmal depolarizing shift" (PDS) by Matsumoto and Ajmone Marsan (Ayala et al., 1973). The synchronous depolarization of a large number of neurons in epileptic focus is associated with a burst of action potentials (Ayala et al., 1973; Menkes, 1990; Crill, 1991; Hopkins, 1993). The summation of these action potentials produces an "interictal spike" of EEG that can be recorded from the surface of the brain or from the skull. The PDS is usually followed by a hyperpolarization potential and neuronal inhibition that corresponds to the slow wave seen on the EEG (Figure 1) (Ayala et al., 1973; Menkes, 1990; Crill, 1991). Studies of the mechanisms of epileptogenesis in brain-slice model systems have indicated that there are three underlying processes which interact with one another and lead to the development of interictal discharge. These include (a) intrinsic burst activity, (b) disinhibition, and (c) excitatory synaptic coupling. Intrinsic membrane excitability may be altered by neuromodulators, injury, genetic, and other factors. Disinhibition releases intrinsic burst generating capacities in population of neurons, and may become an important factor following cortical injury or repetitive activation of inhibitory circuits. Excitatory synaptic coupling is required for evoking intrinsic burst discharges , as well as synchonizing population of

Figure 1. Schematic diagram of relations between cortical discharges and both intracellular and extracellular activity in an epileptic focus. The extracellular recording is through a high pass filter. Isolated interictal discharges and tonic-clonic ictal activity are shown. (From Ayala et al., 1973)



neurons. The contribution of each of these factors to epileptogenesis presumably varies with the type of pathological process and properties of the involved neuronal population (Prince, 1983). The most important unanswered quesion is what is the change in the epileptic focus that periodically releases these "intrinsic epileptic mechanisms" and causes intermittent local spontaneous synchronized firing (Crill, 1991).

4. Treatment of epilepsy by antiepileptic drugs

In order to have a complete control of epilepsy, the appropriate treatment must be selected on the basis of the type of epilepsy and the cause of seizures (Fukuzako and Isumi, 1991). Absence seizure responds well to one group of drugs, and generalized tonic-clonic convulsions are usually adequately controlled by a second. Complex partial seizures tend to be refractory to therapy but may respond to agents in the second group. Infantile spasms and akinetic, atonic, and myoclonic seizures are a group for which therapy is generally unsatisfactory (Rall and Schleifer, 1990). Monotherapy should be recommended in a newly diagnosed epileptic patient because polytherapy does not suppress seizures more effectively, and because drug toxicity and interactions become increasingly common as the number of drugs administered increases (Rall and Schleifer, 1990; Fukuzako and Izumi, 1991).

The choice of the initial and subsequent drugs considered to be appropriate for each seizure type are given in Table 2.

Table 2. Appropriate choice of antiepileptic drugs (From Fukuzako and Izumi, 1991)

Seizure type	Initial drug	Subsequent drug
Partial seizure		
Simple partial	Carbamazepine	Phenytoin
Complex partial	Carbamazepine	Phenytoin, Valproate
Secondarily generalized tonic-clonic	Carbamazepine	Phenytoin, Valproate
Generalized seizure		
Typical absence	Ethosuximide	Valproate
Atypical absence	Valproate	Clonazepam
Myoclonic	Valproate	Clonazepam
Clonic	Carbamazepine	Valproate
Tonic	Carbamazepine	Valproate
Tonic-clonic	Carbamazepine	Valproate, Phenytoin

Amino acid neurotransmitters in epilepsy

Over the years, several amino acids have gained recognition as major neurotransmitter candidates in the mammalian CNS. On the basis of neurophysiological studies, amino acid neurotransmitters are classified into two general classes: excitatory amino acids (glutamic acid, aspartic acid, cysteic acid, and homocysteic acid) and inhibitory amino acids (gamma aminobutylic acid (GABA), glycine, taurine, and β-alanine) The first ones depolarize whereas the latter ones hyperpolarize mammalian neurons in the CNS. (Cooper, Bloom, and Roth, 1991)

1. Excitatory amino acid neurotransmitters : glutamate and aspartate

The excitatory effects of glutamate and aspartate on cerebral cortical cells were first demonstrated by Okamoto in 1951 and Hayashi in 1952 and later on being confirmed by iontophoretic work of Curtis and Watkins in 1960 and 1963. However glutamate and aspartate were not seriously regarded as neurotransmitter candidates until the systematic studies of Krnjevic and Phillis in 1963 in which the appropriate criteria for neurotransmitter action were met by glutamate and aspartate (McGeer, Eccle, and McGeer, 1988).

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^1-pyrroline-5-carboxylic acid (McGeer, Eccle, and McGeer, 1988; Browning, 1992). 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, Eccle, and McGeer, 1988).

Receptors for glutamate and aspartate have been actively investigated. Five distrinct receptor subtypes have been identified. These include the NMDA (N-methyl-D-aspartate) receptor, the kainate receptor, the quisqualate receptor (now called AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor), The ACPD (trans-I-aminocyclopentane-I-3-dicarboxylic acid) receptor, and L-AP4 (L-aminophosphonobutyric acid) receptor which seems to be a presynaptic autoreceptor on glutamate neuron (McGeer, Eccle, and McGeer, 1988; Collingridge and Lester, 1989; Cooper, Bloom, and Roth, 1991; Browning, 1992).

Glutamate receptors (especially the NMDA receptor) are believed to be important in learning and memory through long-term potentiation (LTP), developmental plasticity, epilepsy, and the neurotoxic effects of brain ischemia (Cooper, Bloom, and Roth, 1991; Browning, 1992).

2. Inhibitory amino acid neurotransmitters : GABA and glycine

Once GABA and, to a lesser extent, glycine were identified as major inhibitory amino acid neurotransmitters in the CNS, many studies were initiated to determine their role in seizures. It is now well recognized that the convulsions induced in experimental animal by various agents involve the central nervous synapses using GABA or glycine as their neurotransmitters (Davidoff, 1983).

2.1 GABA

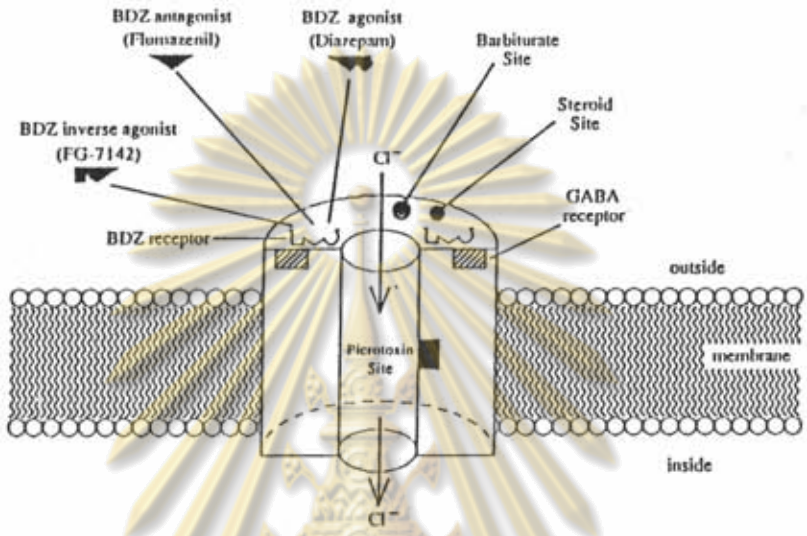
The presence of a large amount of GABA in the brain was reported by Roberts and Frankel in 1950. Subsequently GABA was proved to be an inhibitory neurotransmitter in mammalian brain by Roberts team in 1974 (McGeer, Eccle, and McGeer, 1988).

GABA is synthesized from glutamic acid by the enzyme glutamic acid decarboxylase (L-glutamate decarboxylase, GAD) and degraded to succinic semialdehyde by enzyme GABA transaminase (GABA-T), and then to succinic acid by succinic semialdehyde dehydrogenase (SSADH) (McGeer, Eccle, and McGeer, 1988; Cooper, Bloom, and Roth, 1991; Browning, 1992).

Inhibition through inhibitory synapses can be achieved by either presynaptic inhibition of excitatory neurotransmitter release or by reducing the excitability of postsynaptic cells (Krnjevic, 1991). Two subtypes of GABA receptor, $GABA_A$ and $GABA_B$, have been involved. The two subtypes differ greatly not only in their pharmacological characteristics, but also in a very basic respect (Krnjevic, 1991; Browning, 1992).

$GABA_A$ receptors are stimulated by GABA, muscimol, progabide, SL 75102, 3-aminopropane sulfonic acid, and isoguvacine and are inhibited by the convulsants bicuculline, picrotoxin, penicillin, and pentylenetetrazole (PTZ) (Macdonald and Barker, 1977; Langer et al., 1985; McGeer, Eccle, and McGeer, 1988; Krnjevic, 1991; Browning, 1992). They appear to exist in a macromolecular complex consisting of the GABA recognition site, the chloride channel, picrotoxin binding site, barbiturate binding site, and the benzodiazepine (BDZ) binding site (Figure 2). Activation of $GABA_A$ receptor causes an increase in chloride conductance which usually results in hyperpolarization and inhibition of postsynaptic membrane (McGeer, Eccle, and McGeer, 1988; Cooper, Bloom, and Roth, 1991; Krnjevic, 1991; Browning, 1992). A wide variety of psychoactive drugs, notably barbiturate and benzodiazepine can potentiate the inhibitory action of GABA by a modulation through their respective binding sites on the $GABA_A$ receptor complex (Krnjevic, 1991).

Figure 2. Model of $GABA_A$ receptor complex (from Cooper, Bloom, and Roth, 1991)



$GABA_B$ receptor is reported to be insensitive to bicuculline, 3-aminopropane sulfonic acid, and isoguvacine, but is weakly sensitive to muscimol and stereospecifically sensitive to (-) baclofen. Most of the early studies suggested that $GABA_B$ receptor were primarily presynaptic receptors involved in inhibiting the release of neurotransmitters (such as noradrenaline, glutamate, dopamine , or serotonin); however, they may mediate postsynaptic inhibition as well (Browning, 1992). Unlike the $GABA_A$ receptor, these receptors are not ligand-gate ion channels, but is instead linked to G-proteins and secondary messenger system. Basically, two membrane effects have been attributed to the $GABA_B$ receptors : (1) a decrease in Ca^{2+} conductance (usually a presynaptic effect leading to decreased neurotransmitter release) and (2) an increase in K^+ conductance (resulting in a postsynaptic hyperpolarization) (McGeer, Eccle, and McGeer, 1988; Cooper, Bloom, and Roth, 1991; Krnjevic, 1991; Browning, 1992).

2.1 Glycine

Glycine is a nonessential amino acid which has the simplest chemical structure found in substantial amounts in all mammalian body fluids and tissue proteins. It is believed to function as a neurotransmitter in the spinal cord and brainstem (McGeer, Eccle, and McGeer, 1988; Cooper, Bloom, and Roth, 1991; Browning, 1992).

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 catalysed by the enzyme serine hydroxymethyltransferase (SHMT). Glycine can also be formed from glyoxylate by transamination. (McGeer, Eccle, and McGeer, 1988; Cooper, Bloom, and Roth, 1991; Browning, 1992) However, the radioactive tracer studies suggested that the former pathway was predominant in the brain (Browning, 1992). Though glycine appears to be abundant in the CNS, it is not clear whether the neurons utilizing glycine as a neurotransmitter must synthesize it *de novo* or they accumulate existing glycine (McGeer, Eccle, and McGeer, 1988; Cooper, Bloom, and Roth, 1991; Browning, 1992).

Glycine receptors can be classified into two subtypes according to strychnine sensitivity. The strychnine-sensitive glycine receptor appears to exist in a macromolecular complex which consists of the glycine recognition site, chloride channel, and strychnine binding site (Davidoff, 1983; Browning, 1992). The studies of the distribution of strychnine binding sites show that the density of binding sites is greatest in the grey matter of the spinal cord and decreases progressively in regions more rostral in the neuroaxis (McGeer, Eccle, and McGeer, 1988). Activation of strychnine-sensitive glycine receptor, like the $GABA_A$ receptor, causes an increase in chloride conductance which usually results in hyperpolarization and inhibition of postsynaptic membrane, and this effect can be antagonised by strychnine (Davidoff, 1983 ; McGeer, Eccle, and

McGeer, 1988; Browning, 1992). The strychnine-insensitive glycine receptors are linked to the NMDA excitatory amino acid receptor. This is a high affinity site that appears to increase the action of glutamate at its NMDA receptor. The main effect of glycine is to prevent desensitization of the NMDA receptor during prolonged exposure to agonist. A widespread distribution of strychnine-insensitive glycine binding sites in the brain is similar to that of NMDA receptors. Their endogenous antagonists appear to be the tryptophan metabolite, kynurenic acid and 7-chlorokynurenic acid which is relatively more selective and more potent. (Cooper, Bloom, and Roth, 1991; Browning, 1992)

Amino acid neurotransmitters alteration within epileptic brain

Since GABA is a major inhibitory neurotransmitter in the mammalian CNS, pharmacological manipulation of GABA-mediated neurotransmission has profound effects upon seizures susceptibility in normal laboratory animals; 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). Many studies have revealed the involvement of altered GABA-mediated inhibition in animal models of epilepsy. In some experimentally induced epileptic animals, GABA concentration, 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). On the contrary, GABA concentration and GAD positive neurons were found to be increased in epileptic chicken, gerbils, and epileptic prone rats (Rall and Schleifer, 1990; Horton, 1991). An adaptive response to the decrease in efficacy of GABA receptor and/or an abnormal neuronal circuitry such that GABA neurons inhibit other GABAergic neurons thus causing disinhibition were postulated to be an underlying cause of the increment observed (Rall and Schleifer, 1990; Horton, 1991). In human epileptic brain , there were controversial results on GABA

alteration in epileptic human. Some studies reported that there were significant decrease in GABA concentration, GAD activity, and GABA binding (Lloyd et al., 1981; Lloyd et al., 1985; Tunnicliff and Raess, 1991) whereas no significant differences in all parameters observed were noted by the others (Schmidt and Loscher, 1981; Babb et al., 1989; Tunnicliff and Raess, 1991). Moreover, other inhibitory amino acid, glycine, was slightly elevated in epileptogenic foci (McGeer, Eccle, and McGeer, 1987; Menkes, 1990).

Additionally, excitatory amino acid neurotransmitters also appeared to take part in pathogenesis of epilepsy. An increase in K^+-stimulated release of preloaded [^{3}H]-glutamate and its binding site was reported in genetically epilepsy prone rat (Horton, 1991). Level of glutamine and aspargine but not that of aspartate was markedly increased in photosensitive baboon *Papio papio* seizures (Lloyd et al., 1986). In human, some investigators reported that glutamate and aspartatte levels were increased in epileptogenic in comparison to the nonepileptogenic temporal lobe (Menkes, 1990). However, only glutamate but not aspartate level was reported to be higher in epileptic foci than in control cortex (McGeer, Eccle, McGeer, 1987). Aspartate aminotransferase is also elevated in the epileptic cortex (Menkes, 1990).

Valproic acid

In 1882, valproic acid (2-propylpentanoic acid; dipropylacetic acid, VPA), (Figure 3, 4), was first synthesized in the United State by Burton and used as an organic solvent. In 1963, its anticonvulsant activity was fortuitously discovered when it was used as a solvent in a drug screening program by Meunier and coworkers in France. After its first clinical trial in Europe in 1964, VPA was introduced into armamentarium of antiepileptic therapy. Sodium valproate was introduced as a new antiepileptic drug in France in 1967, in

Holland and Germany in 1968, in United Kingdom in 1973. VPA became available in the United State in 1978. Unlike the other antiepileptic drugs, which are heterocyclic compounds containing nitrogen, VPA is a simple branch carboxylic chain acid and therefore radically different from the other antiepileptic drugs. (Kupferberg, 1982; Penry, 1988; Penry and Dean, 1989; Rogawski and Porter, 1990)

Although initially identified on the basis of its ability to protect against PTZ seizures, valproate was subsequently demonstrated to have a broad spectrum of anticonvulsant activity in a wide variety of animal seizure models (Rogawski and Porter, 1990). VPA is active against tonic and clonic seizures induced by a variety of chemoconvulsants in addition to PTZ, including to bicuculline, picrotoxin, penicillin, quinolinic acid, and strychnine. Furthermore it is also active in the maximal electroshock test as well as in epilepsy-prone animals and kinding model (Swinyard and Woodhead, 1982; Rogawski and Porter, 1990; Davis, Peters, and McTavish, 1994).

Despites an accumulating literature on the metabolic and neurophysiological effects of VPA, the basic mechanism of its action remains obscure. Three major hypotheses on quite different experimental studies exist for the mechanisms of action of VPA.

The first hypotheses suggests that VPA increases GABA level in the brain, thereby exerting its anticonvulsant action by increasing neuronal inhibition. Administration of VPA to experimental animals causes an increase in whole brain and synaptosomal GABA (Simler et al., 1973; Loscher and Vetter, 1985; Rogawski and Porter, 1990). There is an increase in plasma and cerebrospinal fluid (CSF) levels of GABA in long-term drug treatment of epileptic patients and healthy volunteers (Rogawski and Porter,1990; Tunnicliff, 1991). The mechanism by which VPA increases GABA levels is not well understood. The drug has been shown to inhibit several enzymes involved in GABA degradation

including GABA aminotransferase (weakly inhibit), succinic semialdehyde dehydrogenase, and aldehyde reductase. However, on the contrary, several studies have failed to demonstrate that VPA administration lead to inhibition of GABA aminotransferase. In addition, VPA increases the activity of glutamic acid decarboxylase, the enzyme responsible for GABA synthesis (Rogawski and porter, 1990; Tunnicliff, 1991; Davis, Peters, and McTavish, 1994). Although the administration of VPA leads to an increase in brain GABA levels, no real correlation between the anticonvulsant action of VPA and the elevation in cerebral GABA content has been found (Johnston and Slater, 1982; Fromm, 1992).

The second hypothesis suggests that VPA potentiates GABA action. Several investigators using electrophysiological recording techniques have reported that VPA selectively enhanced neuronal responses to exogenously applied GABA (Johnston and Slater, 1982; Rogawski and porter, 1990; Davis, Peters, and McTavish, 1994). However, the concentrations required to potentiate GABA responses in the electrophoretic studies are far higher than the normal therapeutic levels of the drug, indicating that augmentation of GABA-mediated inhibition by the postsynaptic mechanism is unlikely to account for the anticonvulsant action of the drug under normal circumstances (Rogawski and porter, 1990).

The third hypothesis suggests that VPA has a direct membrane effect reducing excitability of neurons. VPA appears to reduce sustained repetitive firing (SRF) through its influence on sodium and potassium conductance (Davis, Peters, and McTavish, 1994). When high concentrations of the drug were applied to isolated *Aplasia* neurons, an increase in membrane potassium conductance developed, leading to an increase in membrane potential (Johnston and Slater, 1982; Davis, Peters, and McTavish, 1994). At concentrations equivalent to therapeutic CSF levels in man, VPA diminished SRF of sodium-dependent action potentials in mouse spinal cord and cortical neurons in culture,

similar to the effects of phenytoin and carbamazepine (McLean and Macdonald, 1986). The relevance of these actions on neuronal potassium and sodium conductance to clinically important anticonvulsant mechanism is unclear (Davis, Peters, and McTavish, 1994).

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, Tunnicliff, 1991).

N-(2-Propylpentanoyl) Urea (VPU) : A monoureide analogue of valproic acid

VPA is one of the major antiepileptic drugs. Although the use of VPA in the treatment of epilepsy has grown during recent years, two major side effects, teratogenicity and hepatotoxicity, have been associated with valproic acid therapy. :-

Transient elevation of liver enzyme activity (alkaline phosphatase, glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT)) have been observed in approximately 11 % of patients receiving VPA (Jeavons, 1982; Davis, Peters, and McTavish, 1994). The studies in rats (Sugimoto et al., 1987) and rat hepatocytes cultures (Jeavons, 1982) indicated that VPA was a dose-related hepatotoxin. Fatal hepatotoxicity, one of the major idiosyncratic effects associated with VPA therapy, seems to occure in correlation with age of the patient and the presence of polytherapy. It is most common in children who are under 2 years of age and receiving multiple drugs but it is rare in older age groups receiving VPA alone (Leppik, 1992; Davis, Peters, and McTavish, 1994).

Studies in animals have shown that VPA exhibits teratogenic effects (Jeavons, 1982). An estimate 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, Peters, and McTavish, 1994). Although the precise biochemical mechanism for the teratogenic effects of VPA and other antiepileptic drugs is unknown, studies suggest that alter folate metabolism and/or interference with folate metabolism by antiepileptic drugs may be partly responsive for the malformation observed (Davis, Peters, and McTavish, 1994).

Together with the fact that VPA is less potent than other three established antiepileptic drugs; phenobarbital, phenytoin and carbamazepine. There is substantially need to develop new derivatives of VPA with higher potency but lower toxicity (Bialer et al., 1994). Numerous derivatives and analogues of VPA have been tested and founded to exert anticonvulsant activity in rodents (Mergen et al., 1991; Elmazar, Hauck, and Nau, 1993; Liu and Pollack, 1994), some of these are the primary amide of valproate (valpromide) and its isomers and analogues, the active metabolites of valproate (i.e. 2-n-propyl-2-pentenoate), and monoester prodrugs of valproate (Bialer et al., 1994).

In an attempt to design active compounds modelled on the partial structure of barbiturate ring and VPA in the same molecule (Figure 4), two monoureide analogues of VPA, n-(2-propylpentanoyl) urea and n-(2-propylpentanoyl) thiourea were synthesized by Boonardt Saisorn and co-worker (Boonardt Saisorn, Chamnan Pantarapanich, and Wicharn Janwitayanuchit, 1992). The synthetic pathways of monoureide and thioureide analogues of VPA are shown in Figure 3., and the proposed structure of VPU is shown in Figure 4.

Figure 3. The synthetic pathways of n-(2-propylpentanoyl) urea and n-(2-propylpentanoyl) thiourea (from Boonardt Saisorn, Chamnan Pantarapanich, and Wicharn Janwitayanuchit, 1992).

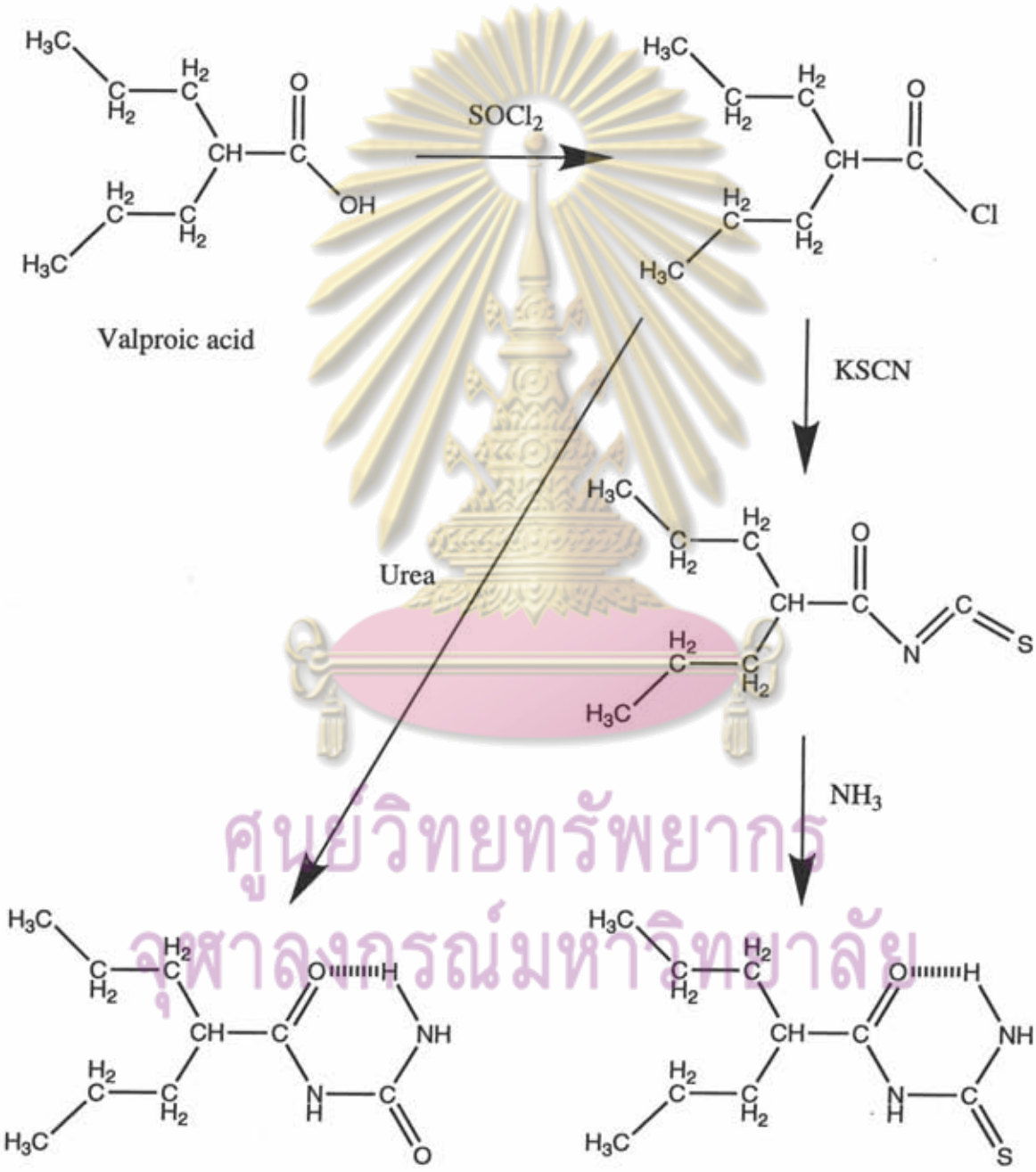
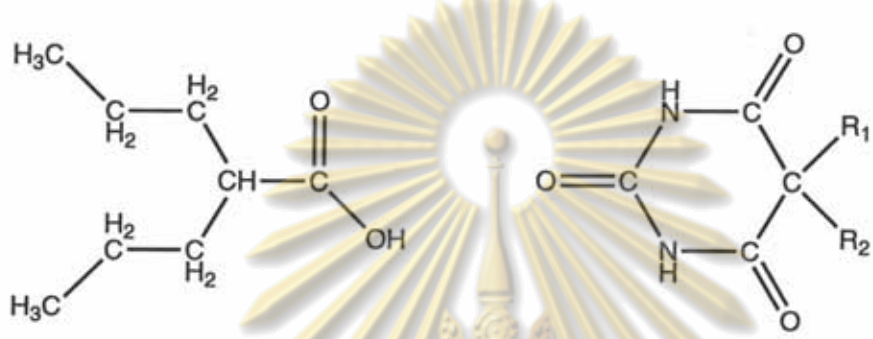


Figure 4. The structures of valproic acid, barbiturates, and propose structure of n-(2-propylpentanoyl) urea showing intramolecular hydrogen bonding (modified from Boonardt Saisorn, Chamna Pantarapanich, Wicharn Janwitayanuchit, 1992.)

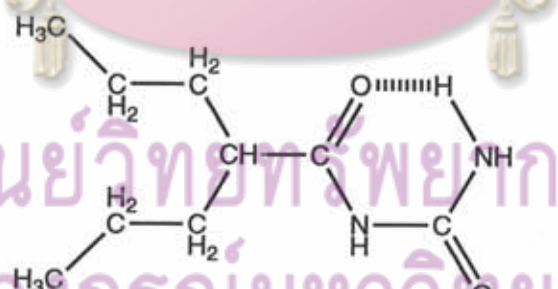


Valproic acid

Barbiturates

Pentobarbital : R1 = ethyl, R2 = 1-methylbutyl

Phenobarbital : R1=ethyl, R2 = phenyl



N-(2-Propylpentanoyl) urea

Preliminary studies of these compounds in this laboratory demonstrated that both of them exhibit anticonvulsant activity which were more potent than VPA. However, the fact that in pararell with its anticonvulsant activity, n-(2-propylpentanoyl) thiourea had also demonstrated a prominent toxicity, therefore only VPU was selected for further investigation in the present studies which aim to determine :-

1) anticonvulsant activity of VPU in relation to VPA in various animal models of epilepsy
2) toxicity in terms of lethality and neurotoxicity of VPU in comparison to VPA
3) effects of VPA and VPU on the levels of excitatory and inhibitory amino acid neurotransmitters in the rat cerebral cortex using microdialysis technique.