Chapter I

Introduction

Epilepsy is a neuropsychiatric disorder with relatively high prevalence and annual incidence rates. According to a considerable number of epidemiological studies from many countries its prevalence and incidence range from 0.15 to 1.95% and from 0.02 to 0.05%, respectively (Zielinski, 1988).

Epilepsy is not a disease but a syndrome of many different cerebral disorders. It may be defined as a disturbance of brain functions of various etiologies characterized by recurrent seizures due to excessive fluctuations in cerebral electrochemical balance associated with a variety of clinical and laboratory manifestations (Fukuzako and Izumi, 1991).

The classification of epilepsy is complicated and can be based on the etiology, pathology, age of onset, clinical seizure, Electroencephalography (EEG) findings, or prognosis. A revised classification of individual seizure types was accepted in 1981 by the General Assembly of the International League Against Epilepsy (ILAE) (Table 1). It has been widely used in the management of epilepsy and serves as a useful tool for guiding decisions about how to treat epilepsy and how to choose among the available antiepileptic drugs (Fukuzako and Izumi, 1991). In general the neurologists use the clinical symptoms and the EEG patterns to classify epilepsy patients into two categories : partial siezures and generalized seizures (Rall and Schleifer, 1990).

Partial siezures have clinical or EEG evident of a local onset. The abnormal discharge usually arises from a portion of one hemisphere and may spread to other parts of the brain during a seizure. Partial seizures can be divided into three types according to generalization and preservation of consciousness. Simple partial seizures do not impair consciousness whereas complex partial seizures do. The preservation of consciousness is generally judged on a basis of an ability to

respond purposefully and to retain memory. The second category, generalized partial seizures, is a spreading of local discharge and an evolvement into generalized seizures (Fukuzako and Izumi, 1991).

Generalized seizures have no evident of localized onset, the abnormal electrical discharges appear simultaneously over the entire cerebral cortex. Many epileptologists infer from this discharge pattern that wide areas of cortex are driven synchronously by diffuse projections from deep cerebral structures, probably in the thalamus or recticular system. Previously, these seizures were subdivided into convulsive and nonconvulsive generalized seizures according to the severity of associated motor disturbances. Nonconvulsive generalized seizures includes absence (petit mal), myoclonic, and atonic seizures. Clonic and tonic-clonic were previously referred to as grand mal seizures (Alldredge,1992).

- An absence seizure (petit mal) consists almost solely of brief lapses of conciousness that last more than 3 seconds. Phenomenologically, a patient suddenly stops moving or shows automatic behavior such as inconspicuous flickering of the eyelids, chewing, or swallowing. During the seizure, there is no loss of postural tone. The patient immediately regains conciousness after the lapse. The intelligence is not usually impaired (Fois, Malandrini, and Mostadrini, 1987). Its onset is usually between the ages of 4 and 12 and rarely persists beyond the ages of 20.

- A myoclonic seizure is bilaterally synchronous, shock-like jerks of limb muscles.

- An atonic seizure is characterized by abrupt loss of muscle tone.

- A clonic seizure is occasionally seen when a generalized tonic-clonic seizure lacks a tonic phase. The postictal phase of this seizure is shorter than in tonic-clonic seizure.

- A tonic seizure represents a rigorous symmetrical tonic contraction of muscles.

- A tonic-clonic seizure (Grand mal) is characterized by loss of conciousness and falling. The body stiffens because of generalized tonic contraction of the axial limb muscles. After the tonic stage, clonic movements occur in the extremities for less than a minute.

In recent papers on the seizure types in adults, almost similar results have been reported as demonstrated in Table 2 (Keränen, Sillanpää, and Riekkinen, 1988). These authors show that partial seizures predominate over generalized seizures and that tonic-clonic seizure (grand mal) is the most frequent type of generalized seizure.

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

Benign partial epilepsy with centrotemporal spikes: The seizure is characterized by a somatosensory onset with unilateral paresthesias involving the tongue, lips, gums, and inner cheeks; followed by unilateral tonic, clonic, or tonic-clonic convulsions which involve the face, lips, tongue, pharynx, and larynx, and leading to speech arrest. Diurnal seizures nerver become generalized and conciousness is preserved. This epilepsy is highly sensitive to antiepileptic drugs and disappears by the age of 20 without any treatment (Fukuzako and Izumi, 1991).

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

Febrile convulsion: It occurs in children with predisposing factors only when a feverish illness occurs during the critical age period (before the age of 5). In children with some risk factors for recurrence, prophylactic antiepileptic medication is needed. Valproate may be appropiate beacuse it has few effects on cognitive function and behavior (Fukuzako and Izumi, 1991).

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

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

Proper diagnosis and classification of epilepsy are essential for the complete control of seizures. Misdiagnosis of nonepileptic seizures as epileptic results in treatment failure. The best treatment must be selected according to the type of epilepsy and the cause of seizures. Drug therapy is the main form of treatment of epilepsy. Monotherapy should be recommened in a newly diagnosed epileptic patient beacuse polytherapy does not suppress seizures effectively and drug toxicity and interactions become increasingly common as the number of drugs administered increases. Antiepileptic drugs of choice have shown in table 3 and Valproate has been widely used in the treatment of several types of seizures (Alldredge,1992).

Table 1
International Classification of Epileptic Seizures in 1981 (Fukuzako and Izumi, 1991)

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 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 absence seizures
- B. Myoclonic seizures
- C. Clonic seizures
- D. Tonic seizures
- E. Tonic-Clonic seizures
- F. Atonic seizures

III. Unclassified epileptic seizures

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

Table 2
Distribution of seizure types in 1,005 epileptic patients with classificable seizures (Keränen, Sillanpää , and Riekkinen, 1988)

Seizure types	No. of cases	%
Partial Seizures	682	67.9
Simple partial	92	9.2
Complex partial	280	27.9
secondarily generalized	310	30.8
Generalized seizures	323	32.1
Absence seizures	17	1.7
Myoclonic seizures	13	1.3
Tonic or Clonic seizures	7	0.7
Tonic-clonic seizures	283	28.1
Atonic seizures	3	0.3
Total	1,005	100.0

Modified from Keränen, Sillanpää, and Riekkinen,1988

Table 3
Antiepileptic drugs of choice based on seizure classification (Alldredge,1992).

	Partial seizures	Generalized Tonic-Clonic seizures	Absence seizures	Myoclonic seizures
Drug of choice	Cabamazepine Phenytoin	Valproate Carbamazepine Phenytoin	Ethosuximide Valproate	Valproate
Alternatives Primary	Phenobarbital Valproate Primidone	Phenobarbital Primidone	Clonazepam	Clonazepam
Secondary	Clorazepate		Acetazolamide	

From Clinical Pharmacy and Therapeutics 5th edition, Section 13 : Neurologic disorder, Chapter 48 : Seizure disorders

Mechanism of epileptogenesis

Although a major seizure may involve nearly the entire central nervous system (CNS) but the simplest form a focal seizure represents an abnormality of function in only a small portion of the brain. It is called epileptic focus. To study the machanism of the epileptogenesis, numerous agents have been used to induce focal seizures in experimental animals. One of the most common and effective agent is to apply a crystal of penicillin directly to the surface of the cortex (Ayala et al., 1973) and the change characteristics can be obtained in the EEG. Typically high voltage sharp negative waves appear intermittently at the center of the focus within 5 minutes. These are called 'Interictal spikes' because they appear between ictal episodes and a spike-like configuration on the EEG paper (Ayala et al., 1973). These focal cortical discharges are similar to those presented in clinical seizure disorders.

The interictal discharges are associated with an intracellularly recorded-prolonged persistent depolarization, and often superimposed action potentials (Figure 1)(Lothman and Collins, 1984). This cellular response is called the paroxysmal depolarization shift or PDS. The PDS often inactivates the neuronal action potential generating mechanism. Following the seizure, the cell hyperpolarizes and excitability is decreased during the postictal period. These cellular events are present in each of the experimental models of epilepsy (Crill, 1991).

A development of paroxysmal discharge in the epileptic cortical focus is on the basis of neuronal circuit If a balance between excitatory and inhibitory circuit is interfered by epileptogenic agents, the paroxysmal events will occur (Crill, 1991). A penicillin, for instance, plays a role by decreasing the inhibitory circuit activity by acting as an antagonist of GABA (Hochner, Spira, and Werman,1976 ; MacDonald and Barker, 1977).

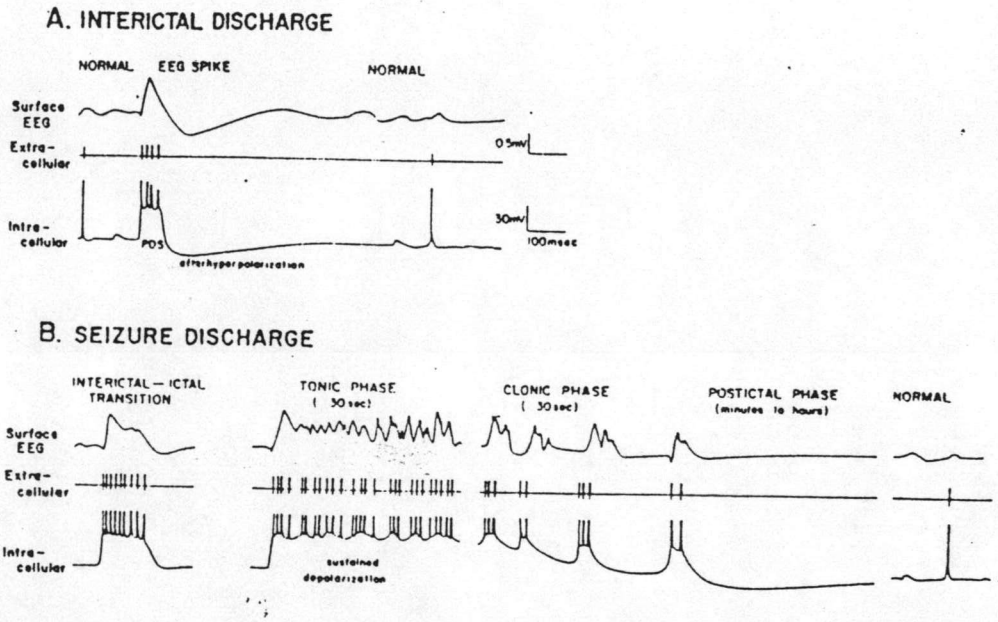


Figure 1. Drawings of electrocorticogram (Top trace), extracellular unit recording (Middle trace), and intracellular recording (Bottom trace) during interictal (A) and ictal behavior (B) (Lothman, and Collins, 1991)

GABA is a neurotransmitter that inhibitory cells release in all parts of the vertebrate brain. It is formed in the brain from L-glutamic acid by an α-decarboxylation reaction. This reaction is catalyzed by the enzyme L-glutamic acid decarboxylase (GAD). The major pathway of GABA degradation is via transamination with α-ketoglutarate to form L-glutamic acid and succinic semialdehyde. This reaction is catalyzed by an GABA-aminotransferase (Hearl and Churchich, 1984).

The inhibitory synapses can operate in two general ways. The reduction of a release of excitatory neurotransmitter through a pre-synaptic action and the excitability of post-synaptic cells via the post-synaptic inhibition are counted. GABA, infact, possesses both pre-synaptic and post-synaptic inhibitory effects (Krnjevic,1991). Two types of the GABAergic receptor are classified, i.e., $GABA_A$ and $GABA_B$ receptors. Both of them provide pre-synaptic or post-synaptic inhibitory effects. In post-synaptic inhibition, GABA is able to increase the membrane conductance for an ion which enables cells to be hyperpolarized. $GABA_A$ and $GABA_B$ inhibition act by raising Cl^- and K^+ conductance, respectively. In pre-synaptic inhibition, the machanism is not explicitly clear, however, the assumption was made that $GABA_A$ was involved with the increase of Cl^- conductance and of $GABA_B$ with a direct depression of terminal Ca^{2+} current (Krnjevic,1991).

Most of anticonvulsant drugs or convulsive agents in epileptic models are related to $GABA_A$ receptor. The studies of $GABA_A$ receptor show that this receptor is a protein complex consisting of three receptor proteins, i.e.,chloride channel protein of barbiturate site, benzodiazepine (BDZ) receptor and GABA receptor proteins (Figure 2) (Polc, et al.,1982). Each of the receptor proteins has its own specific action to control the chloride channel. The GABA receptor protein has direct effects on the control of chloride channel opening, whereas the BDZ receptor protein and the barbiturate site show their actions by an alteration of

GABA affinity to the GABA receptor protein or an ability of opening the chloride channel (Krnjevic,1991).

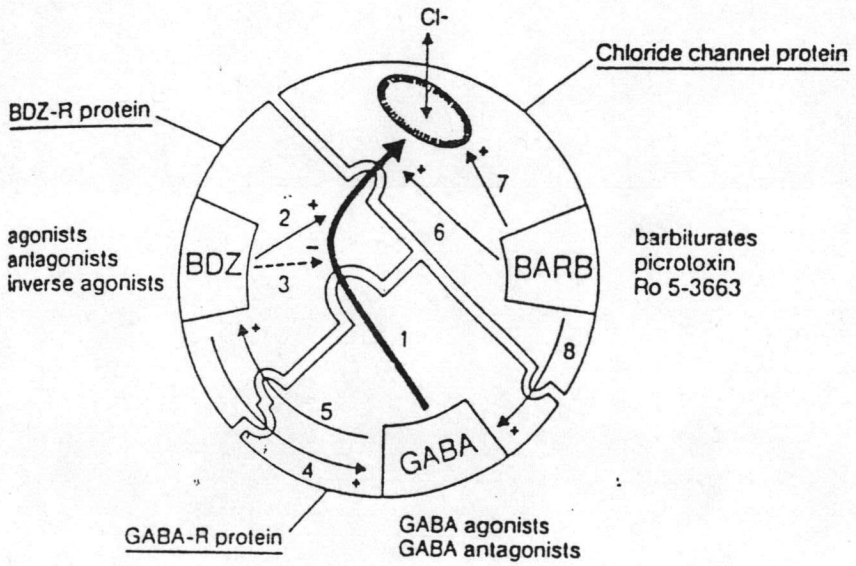


Figure 2. The model of $GABA_A$ receptor protien complex (Polc, et al.,1982)

The agents that have the direct actions on the GABA receptor protein is classified as true $GABA_A$ agonist or antagonist. Muscimol and progabide are true $GABA_A$ agonists and they have been used as an anticonvulsant drug (Löscher ,1982 ; Zivkovic, Lloyd and Bartholini, 1985) while penicillin, bicuculline, and PTZ are true $GABA_A$ antagonists which induce seizures (Curtis, et al., 1970 ; MacDonald and Barker, 1977). The effect of the agonists has been produced by direct changing in chloride current as these ligands bound to its receptor and, hence, changing the opening properties of the chloride channel. The increased concentration of GABA can produce an increase of channel opening and burst frequencies and durations without altering the channel conductance (Figure 3) (MacDonald, Roger, and Twyman, 1989) so that the inhibitory effects of $GABA_A$ receptor will be increased. These may explain their anticonvulsant properties. On the contrary, the binding of $GABA_A$ antagonists would certainly induce seizure.

The other protein binding sites such as the BDZ receptor and the barbiturate receptor sites can also control the chloride channel by a modulation of effect of GABA on the $GABA_A$ receptor protein. Binding to the BDZ receptor site by BDZ agonist enhances the effects of GABA to increase chloride current. In contrast, the binding of BDZ antagonist to BDZ receptor reduces the effect of GABA thus the chloride current is consequently reduced. The mechanism of BDZ modulators is believed to be an alteration of the chloride channel opening's frequency (Figure 4) without changing in chloride conductance (Twyman and MacDonald, 1991). The BDZ agonists (diazepam, clonazepam, lorazepam, etc.) have been used as anticonvulsant drugs because of their ability in enhancing the GABA effects while BDZ antagonists (β-carboline, flumazenil, etc.) show convulsive effects due to negative modulations on $GABA_A$ receptor. In a similarity to the action of BDZ, another anticonvulsant, barbiturate agonists (pentobarbital, phenobarbital, etc.) also enhance the GABA effects and being used as anticonvulsant drugs while their antagonist (picrotoxin, Ro 5-3663) are convulsants. Their mechanisms, however, behave in a different manner. The barbiturate modulators control the chloride

channel activity by an alteration of the channel opening duration (Figure 4) such that the change in chloride current occurs. The change in chloride channel opening frequency and conductance, nevertheless, have not been detected (Twyman, and MacDonald ,1991).

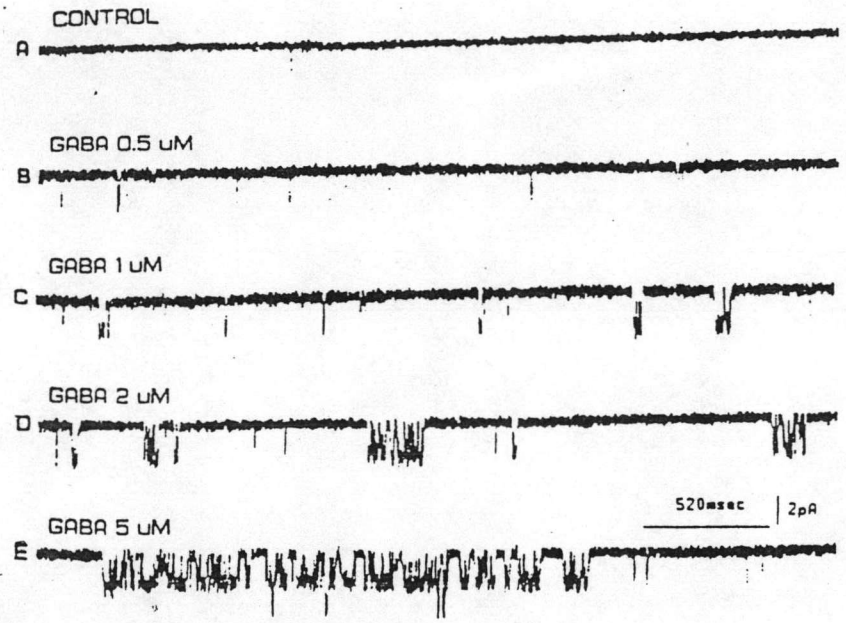


Figure 3. Single channel GABA-evoked currents were concentration-dependent. Rare, spontaneous openings were observed before the application of GABA (A). Application of GABA (0.5-5 uM) produced a concentration-dependent increase in openings and burst frequencies (MacDonald, Roger, and Twyman, 1989).

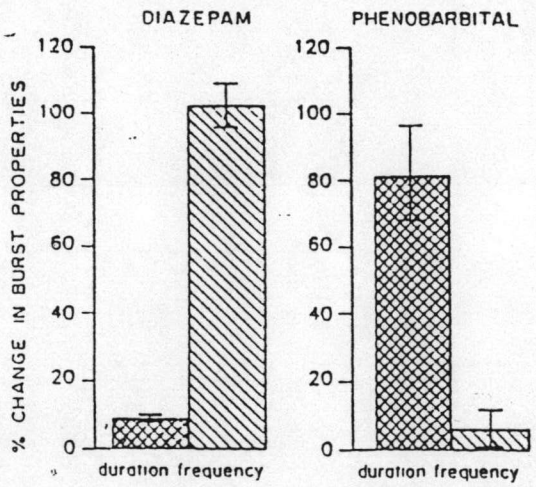


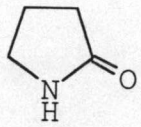
Figure 4. Histograms showing different effects of diazepam and phenobarbital on the properties of GABA evoked bursts. In the presence of diazepam, GABA-evoked burst were more frequent but were not prolonged. In contrast, in the presence of phenobarbital, burst frequency was not changed, but burst duration was prolonged (Twyman and MacDonald, 1991).

GABA activity and anticonvulsant effects

The previous section has described the important roles of GABA in CNS inhibitory circuit neurons that hypofunctions of GABA may results in the genesis of seizures. Many antiepileptic drugs are GABA agonists. These agonists are either true $GABA_A$ agonists or $GABA_A$ modulators. Both types of agonists increase the inhibitory effects of $GABA_A$ receptor which ensure their antiepileptic activities.

There are several methods to enhance GABA activity in the brain. One of them is to increase brain GABA levels which is commonly achieved by an administration of GABA but the fact that GABA crosses blood brain barrier very poorly (Chase and Walters, 1976) leads to the development of a large number of GABA derivatives such as alkyl esters of GABA, aliphatic and steroid esters of GABA, etc. These GABA derivatives are more lipophilic than GABA thereby can easily penetrate through blood brain barrier (Shashoua, et al.,1984). By means of hydrolytic enzymes in the brain these derivatives can be further hydrolyzed to GABA.

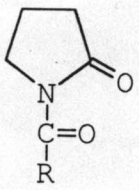
There are another attempt to increase the level of GABA in brain by the use of 2-Pyrrolidinone as GABA prodrug (Figure 5). 2-Pyrrolidinone, the lactam of GABA, has the advantages over GABA due to its ready penetration and hydrolyzing into an active form. However, the attempt to increase the level of brain GABA concentration with a single large dose of 2-Pyrrolidinone have not been proved successfully. The failure can be explained by a slow hydrolysis of 2-Pyrrolidinone into an active form, GABA as shown in Figure 6 (Callery, Stogniew and Geelhaar, 1979). However, The tight amide bond of 2-Pyrrolidinone may be weakened by an introduction of an acyl function at the 1-position (Figure 5). and the lipophilicity of drug can also be controlled by the chain length of the acyl function (Sasaki, et al.,1991). The studies of anticonvulsant activity of various 1-acyl-2-pyrrolidinone derivative have demonstrated that anticonvulsant activity was exhibited by both GABA releasing and non GABA releasing derivative (Sasaki, et al.,1991).



2-pyrrolidinone

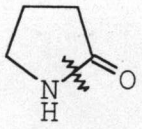
$H_2N-CH_2-CH_2-CH_2-COOH$

GABA



1-Acyl-2-pyrrolidinone

Figure 5. The structure of 2-pyrrolidinone, GABA, 1-Acyl-2-pyrrolidinone



Hydrolysis

$H_2N-CH_2-CH_2-CH_2-COOH$

2-pyrrolidinone

GABA

Figure 6. Hydrolysis of 2-pyrrolidinone to GABA

Valproic acid

Valproic acid (VPA ; 2-propylpentanoic acid ; or dipropylacetic acid) was synthesized by Burton in 1882 and shown to have anticonvulsant properties by Meuneir et al. in 1963. The VPA was licensed for use as an anticonvulsant in the United States in 1978 (Johnston, 1984). Currently, the VPA has been widely used as a broad spectrum anticonvulsant that is effective agianst a variety of seizures in human (Table 3) and in experimental animal models.

The mechanism of action of VPA remains obsecure. The recent findings have suggested the involvement of both GABA and ionic processes related to the intrinsic mechanism of epilepsy at the cellular level (Morre, et al.,1984).

On the basis of GABA hypothesis, VPA has been considered to play a critical role in increasing the GABA content in brain, hence, the anticonvulsant activity. It has been suggested that the VPA increases the brain GABA by an inhibition of its degradation (GABA-T inhibition) or an activation of its synthesis (increasing GAD activity), but these effects do not usually appear to be related to its antiepileptic action (Kerwin, Olpe, and Schmutz, 1980). There was no simple relationship between the elevation of brain GABA and an anticonvulsant effect as concluded by Johnston and Slater (1982). They postulated that the VPA might act by limiting the spread of seizure activity, rather than directly suppress the seizure focus. This might explain its lower effectiveness in complex partial seizures with focal temporal spikes in human (Johnston and Slater, 1982).

Another mechanism of action of VPA on the GABA hypothesis is that VPA may rather act at the postsynaptic membrane of GABAergic synapse. The administration of valproate by microiontophoresis potentiates the inhibitory action of GABA on neuron in the brain (Gent and Phillips,1980), in spinal cord culture (MacDonald and Bergey, 1979), and in cuneate fiber preparations (Harrison and Simmonds,1982). This action of valproate appears to be specific to GABA as the effects of glycine and glutamate are not potentitated (MacDonald and Bergey, 1979). Valproate neither inhibits nor enhances the binding of GABA or BDZ to

their receptors (Löscher, 1980 ; Ticku and Davis, 1981), so the molecular basis of its ability to potentiate the effects of GABA is unknown.

There was another hypothesis that mechanisms of actions did not involve with GABA but with ionic process related to the intrinsic mechanism of epilepsy at the cellular level. Single-cell studies carried out in hippocampus and cortex indicate the existence of cells that generate bursting activity during interictal EEG spikes. Nueronal excitability in these cells appears to be regulated by a delicate balance between inward currents carried by Na^+ and Ca^{2+} and outward currents mediated by K^+. Any action that reduces the exit from cells of K^+ ions or increases the entry of Na^+ or Ca^{2+} ions can produced bursting activity. The first spikes of the burst are produced by influx of Na^+, and the depolarization and burst activity are then ended by a Ca^{2+} activated efflux of K^+ ions (Schwartzkroin, 1980 ; Prince, 1983). The role of K+ efflux in the termination of bursting activity has stimulated an analysis of the effects of antiepileptic drugs on this parameter. The effects of valproate on K^+ conductance have been studies in nonmammalian species. The results of these studies with valproate confirm a direct effect on membrane activity that is independent on chloride conductance, but related to K^+ conductance (Hackman, Grayson, and Davidoof, 1981 ; Slater and Johnston , 1978).

It appears that VPA propably has more than one mechanism of action to exert its ability as a broad spectrum anticonvulsant.

N(2'-propylpentanoyl)-2-pyrrolidinone

VPP is a new synthetic chemical that has been proposed to possess an antiepileptic activity. This agent is a derivative of 1-acyl-2-pyrrolidinone. It was synthesized by reacting pyrrolidinone sodium with valproyl chloride as illustrated in Figure 7 (Wicharn Janwitayanuchit .,1992).

The VPP consists of two parts. One is VPA and the other is 2-pyrrolidinone. Each of them exhibits antiepileptic property as previously described. Expected antiepileptic activity of this compound, Therefore, has been built on the assumption that VPP is a prodrug which can be degraded into active metabolites, i.e. VPA and GABA (Figure 8) in brain.

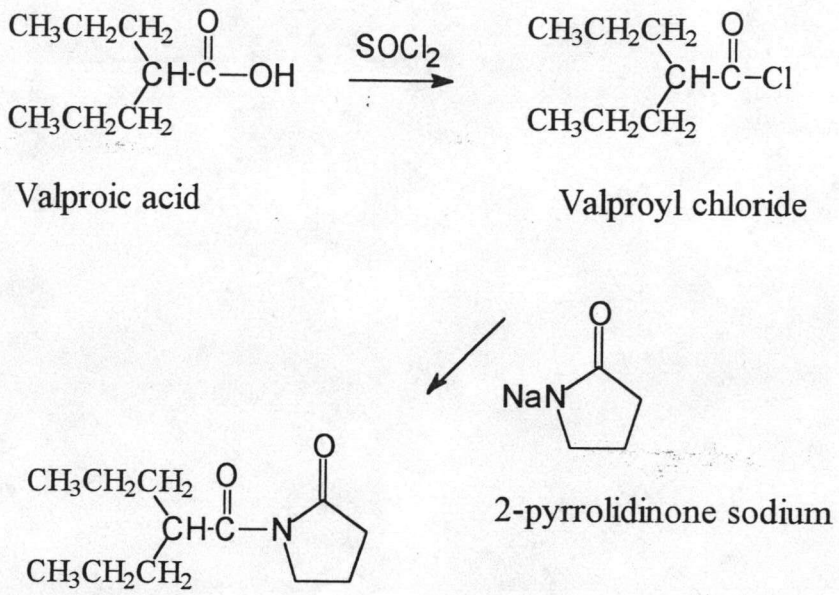


Figure 7. The synthesis scheme of VPP (Wicharn Janwitayanuchit .,1992)

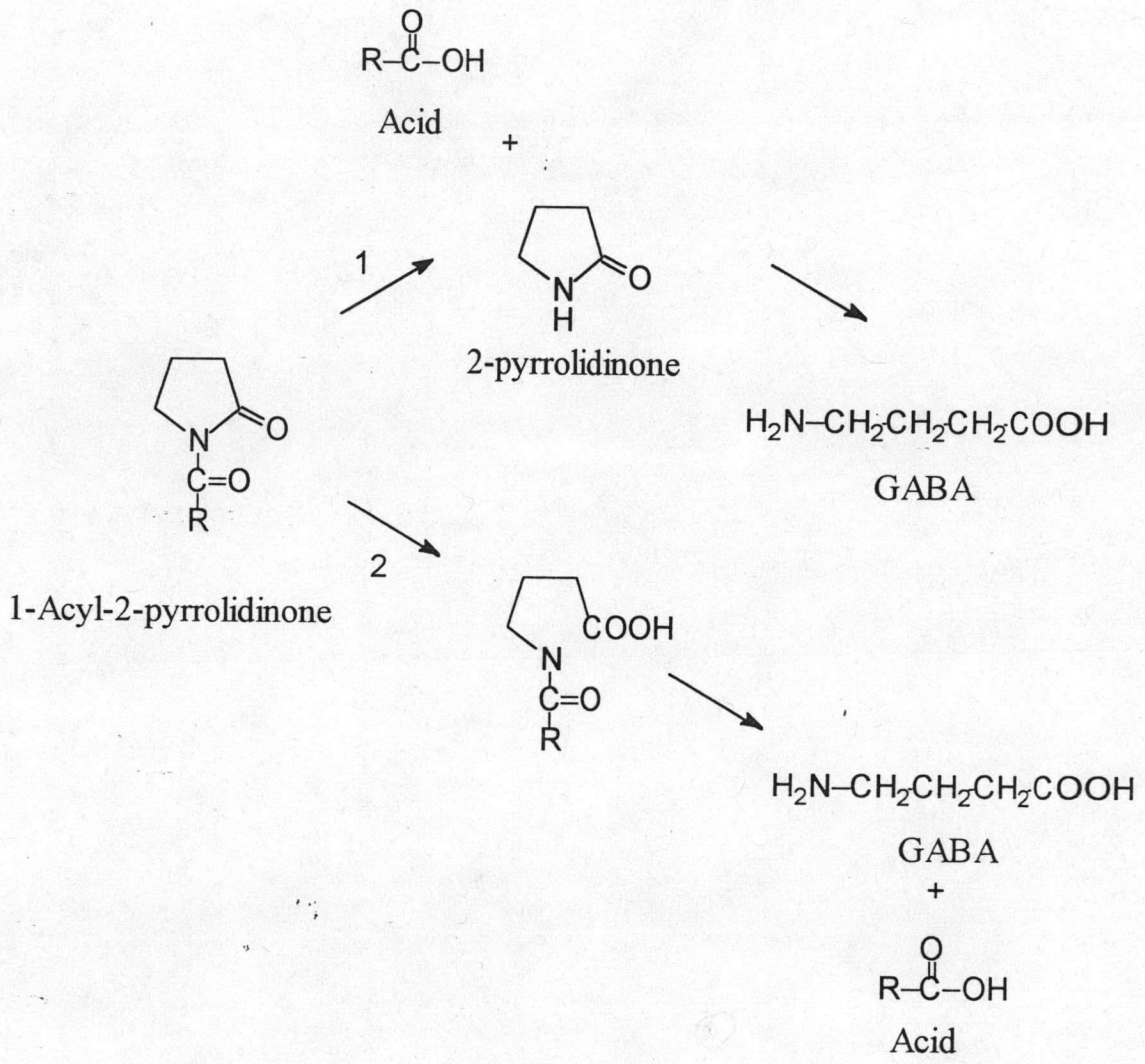


Figure 8. The possible pathways of hydrolysis of 1-Acyl-2-pyrrolidinone to GABA

This thesis aims to study;

1 Antiepileptic activity of VPP in experimental animal using MES and PTZ seizure models.

2. Degradation of VPP in liver and brain homogenate of the rats.

3. Effect of VPP on GABA levels in the cortex of rats by microdialysis method.