Chapter II

Background Information

Epilepsy

Epilepsy can be defined as a disturbance of brain functions of various etiologies characterized by recurrent seizures due to excessive fluctuation in cerebral electrochemical balance associated with a variety of clinical and laboratory manifestation (Fukuzako and Izumi, 1991).

1. Etiology.

1.1 Pathology causes

Epilepsies are caused by virtually any major category of serious disease or disorder of humans. It can be resulted from congenital malformations, infections, tumors, vascular diseases, degenerative diseases, injury or heredity. In more than three-quarters of patients with epilepsy, the seizures begin before the age of 18 years. The reason for this age of onset is not clear (Porter, 1993). Recurrent seizures are thought to be resulted from a genetic predisposition which appears to be the most significant in patients with various primary epilepsies (Menkes, 1990; Hopkin, 1993).

1.2 Biochemical causes

The neurotransmitter basis of epilepsy has been suggested to be due to either an excess of excitatory mechanisms or a loss of inhibitory neurotransmission (Lloyd et al., 1981). If reduction of GABAergic and glycinergic transmission is a causative factor in epileptogenesis (Davidoff, 1983), there are little data to suggest that glycine which exerted its major effect in spinal cord is a transmitter in the cerebral cortex. Thus, the mechanism of antiepileptic drugs, should it be any, in this aspect, is likely to involve with an enhancement of GABAergic transmission (De Deyn and Macdonald, 1989).

The amino acid content of nerve ending isolated from brain tissue at the onset of seizures was characterized by a significant increase in glutamate levels and a decrease in Gamma-aminobutyric acid (GABA) concentration. Smaller and less uniform changes occurred in the contents of aspartate (decrease) and glutamate (increase). The contents of serine and taurine were not affected by any of the convulsant agents (Geddes and Wood, 1984).

Primary defect in epilepsy manifested as an excessive glutamate-mediated transmission. This may occur either as an increased release of transmitter, possibly as a result of failure of inhibitory mechanisms, or as a consequence of the postsynaptic glutamate receptors becoming hypersensitive. However, many theorists believe that epilepsy results from the hypofunction of inhibitory transmitters such as GABA. (Davies and Richens, 1993).

In some experimentally induced epileptic animals, GABA concentration, Glutamic acid decarboxylase activity and GABA binding were markedly

decreased and thus probably contributed substantially to the epileptic discharges (Horton, 1991). Not all animal models for epilepsy have been related to GABA neuron function (Lloyd et al, 1981). In human epileptic brain, there were controversial results on GABA alteration in epileptic human. GABA concentrations in human epileptic cortex are normal or perhaps slightly increased. The activities of GAD and GABA-T are normal in whole brain and synaptosomes and GABA binding is increased (Tunnicliff and Raess, 1991).

Glycine levels have been found to be elevated in epileptic foci. Significant increases in the concentrations of glutamate and aspartate have been found in the epileptogenic as compared with the nonepileptogenic temporal lobe. Brain norepinephrine levels are low in several species of epileptic animals, and a decrease in brain norepinephrine concentration increases their seizure susceptibility (Menkes, 1990).

Seizures are accompanied by profound changes in cerebral metabolism and, conversely, metabolic changes in the brain can give rise to seizures (Goldensohn et al., 1984). In experimental animals, a major motor seizure induced by electroshock results in a rapid decrease in brain levels of Adenosine triphosphate (ATP), glucose and glycogen, and concomitant accumulation of lactate. This indicates that the rate of utilization of high-energy phosphate during seizure activity is greater than its rate of production (Collins and Ferrendilli, 1978).

2. Biochemical Mechanism of Seizure

Experimentally, two major biochemical mechanisms are recognized: (1) defects in the membrane regulation of ion fluxes in some forms of epilepsy; and (2)

impaired synaptic transmission resulting in the depolarization of neurons with resulting epileptogenic discharges. Inhibition of Na^+- K^+ ATPase, the Na^+- K^+ pump results in seizures by allowing an increase in intracellular sodium and a decrease in potassium within the cell (Goldensohn et al, 1984).

In other experiments, it was found that Glia cell passively transferred excess K^+ away from a region of excitation and help maintain extracellular homeostasis. They could play a role in preventing the onset of seizure activity locally since an increase in extracellular K^+ depolarizes the neuronal membrane, and excessive neuronal depolarization is epileptogenic (Goldring, 1978).

Alterations in the functional status of GABAergic neurons have been implicated in a large number of animal models for epilepsy, as well as in the mechanism of action of several antiepileptic drugs. Thus, inhibition of GABA synthesis or blockade of GABA receptors or of GABA receptor-mediated changes in chloride ion flux rapidly induces seizures (Lloyd et al., 1981).

3. Classification of Seizures

The classification of epilepsy is complicated and can be based on the etiology, pathology, age of onset, clinical seizure, electroencephalogram (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) (Porter, 1993). Epileptic seizures are fundamentally divided into two groups of partial and generalized (Rall and Schleifer, 1990). The symptoms of any particular epileptic attack depend upon the presumed function of the region of the brain which is being interrupted by the excessive neuronal discharges (Hardin,1978)

Generalized seizures have no evidence of localized onset, the abnormal electrical discharges appear simultaneously over the entire cerebral cortex. Many epileptologists infer from this discharge pattern that wide areas of cortex are driven synchronously by diffuse projections from deep cerebral structures, probably in the thalamus or reticular system (Alldredge, 1992; Porter, 1993). They include tonic-clonic attacks (grand mal-periods of tonic rigidity followed later by massive jerking of the body) and absences (peptit mal changes in consciousness usually lasting less than 10 sec.) (Neal, 1987).

Partial seizures have clinical or EEG evidence of a localized onset . The abnormal discharge usually arises from a portion of one hemisphere and may spread to other parts of the brain during seizure (Fukuzako and Izumi, 1991; Porter, 1993). It may be limited to clonic jerking of an extremity (Neal, 1987).

4. Therapy

4.1 Non-Drug Therapy

A non drug therapy has been tried, including diet, biofeedback, cerebellar stimulation and surgery. Of these, only surgery has gained widespread acceptance. But, less than 10% of the epileptic population meet these criteria. Thus, for most epileptic patients, the major therapy for seizures is drug therapy (Burnham, 1989).

4.2 Drug Therapy

The best treatment must be selected according to the type of epilepsy and the cause of seizures. In choosing the most appropriate antiepileptic

drug for an individual patient, a clinician must assess the efficacy and safety of the various antiepileptic drugs in terms of the patient clinical profile (Wilder and Rangel, 1988).

The most effective drugs for partial and secondary generalized epilepsies are phenobarbital, phenytoin and carbamazepine. Phenobarbital was the first modern anticonvulsant. It is still one of the cheapest and safest anticonvulsants but this drug causes sedation at the therapeutic dose levels. Phenytoin is an older drug that is still the drug of choice for tonic clonic seizure which replaced phenobarbital because of its lack of sedation but its disadvantages are annoying side effects (acne, gingival hyperplasia) which may occur at therapeutic dose. Carbamazepine is as effective and usually as nonsedating as phenytoin. However, its disadvantages are expensive cost and long half life of 30-60 hours, though serious toxicity is not frequent (transient mild depression of leukocyte count). Primidone, resembles phenobarbital, is long acting and fairly safe like phenobarbital. It is sedating and also declining in popularity (Wilder and Rangel, 1988; Burham, 1989; Rall and Schleifer, 1990).

The most effective drugs for generalized epilepsies are clonazepam, ethosuximide and valproate. Ethosuximide is the current drug of choice for absence seizures due to the absence of sedative effect but it may cause gastrointestinal disturbance, fatigue and other effects. Clonazepam is a drug of last resort because tolerance to its effects usually develops after 1-6 months of administration (Wilder and Rangel, 1988; Burham, 1989; Rall and Schleifer, 1990). Valproic acid is one of antiepileptic drugs of choice that has been widely used in the treatment of several types of seizures (Alldredge, 1992). It is highly effective in controlling generalized and partial seizure, particularly as monotherapy (Penry, 1988).

Amino Acid Neurotransmitters

Over the years several amino acids have gained recognition as major neurotransmitter candidates in the mammalian central nervous system (CNS). On the basis of neurophysiological studies, amino acids have been separated into two general classes: excitatory amino acids (glutamic acid, aspartic acid, cysteic acid, and homocysteic acid), which depolarize neurons in the mammalian CNS; and inhibitory amino acids (GABA, glycine, taurine, and β-alanine) which hyperpolarize mammalian neurons (Cooper, Bloom and Roth, 1991).

1. Inhibitory amino acid neurotransmitters

High concentration of GABA is found in all brain regions and spinal cord. It has been estimated that GABA was the transmitter at 30% of all the synapses in the brain (Davies and Richens, 1993). The receptor for GABA has been classified into $GABA_A$ and $GABA_B$ types and their action are mediated by different molecular mechanisms (Kuriyama, Hirouchi and Nakayasu, 1993).

$GABA_A$ receptors are stimulated by GABA, muscimol, and isoguvacine and are inhibited by the convulsant bicuculline (competitively) and picrotoxin, $GABA_A$ receptors opens the chloride channel in the GABA/benzodiazepine complex and this allows an influx of Cl^- which leads to hyperpolarization of the postsynaptic neurons and consequently an inhibition of firing. As this mechanism involves ionic fluxes, it has been termed ionotropic transmission. The $GABA_B$ receptors are sensitive to baclofen. A linkage of these $GABA_B$ receptors to a G protein(s) is the most probable transduction process. Activation of $GABA_B$ receptors leads to a decrease in Ca^{2+} conductance (influx) and/or an increase in K^+ conductance which would lead to

hyperpolarization of the neurons. Either of these possibilities would decrease transmitter release (Browning, 1991)

GABA is synthesized from glutamic acid by the enzyme 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) Succinic semialdehyde may also be then converted by enzyme aldehyde reductase to gamma hydroxybutyrate(GHB), a compound which, has been shown to cause absence-type seizure (Snead, 1988)

Glycine is an inhibitory neurotransmitter in the mammalian spinal cord. Like GABA, its actions seem to be mediated through a ligand-gated chloride channel and is antagonized by strychnine. The release of glycine is prevented by tetanus toxin. Both strychnine and tetanus toxin are convulsants (Neal, 1987). Glycine receptors can be classified into two subtypes according to strychnine sensitivity. The strychnine-insensitive glycine receptors are linked to the N-methyl-D-aspartate (NMDA) excitatory amino acid receptor to increase the action of glutamate at its NMDA receptor (Cooper et al., 1991).

2. Excitatory amino acid neurotransmitters

Glutamate and aspartate are excitatory amino acid neurotransmitters. Glutamate is found in higher concentrations than any other free amino acid in the CNS , being three or four times higher than taurine, or aspartate, and six times higher than GABA (Browning, 1991). Glutamate plays an important function in the detoxification of ammonia in the brain. It is an important building block in the synthesis of GABA as well as in the synthesis of proteins and peptides including

glutathione (Cooper et al., 1991). Glutamate and aspartate may contribute to tissue damage within the CNS in a variety of disorders including epilepsy, neurodegenerative diseases and cerebral ischemia. At least three receptor subtypes for excitatory amino acids have been classified on the basis of their respective sensitivity to the agonists NMDA, Quisqualate and kinate (Faden, Ellison and Noble, 1990).

Valproic acid

Valproic acid (VPA; 2-propylpentanoic acid or dipropylacetic acid) was synthesized by Burton in 1882 and shown to have anticonvulsant properties by Meunier et al. in 1963. VPA was introduced as the 16th primary antiepileptic drug marketed and licensed for use as an anticonvulsant in the United States in 1978. Unlike the other antiepileptic drugs which are heterocyclic compounds containing nitrogen, valproate is a simple branched carboxylic chain acid and therefore radically different from the others antiepileptic drugs (Penry, 1988; Davis, Peters and McTavish, 1994).

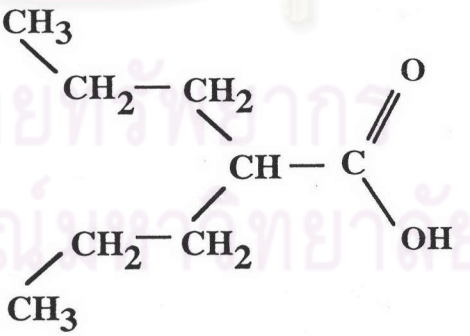

Figure 1 The structure of 2-Propylpentanoic Acid (Valproic Acid ;VPA)

1.1 Pharmacological Effects

Valproate is used primarily for the treatment of generalized seizures (Johnson, 1984). It has a board spectrum of antiepileptic effects (Penry, 1988).

Although valproate is less effective for the treatment of partial seizures, it appears to be as effective as phenytoin or carbamazepine (Richens and Perucca, 1993) and phenobarbital in the treatment of mixed type of generalized and partial seizures. It is clearly nonsedating and equally effective as ethosuximide in the treatment of absence seizures (Davis et al., 1994).

As the broad spectrum of activity of valproate has been uncovered, valproate offers a significant treatment alternative for many patients, especially those with partial seizures and psychiatric disorders (Penry and Dean, 1993). Several investigators have proposed its use in the treatment of anxiety, alcoholism and mood disorders, although these indications require further clinical studies (Zaccara, Messori and Moroni, 1988).

The results of animal studies showed that valproic acid possessed certain anticonvulsant properties. The drug protects dogs, cats, rats, rabbits, and mice against seizures induced by maximal electroshock (MES), pentylenetetrazole (PTZ), bicuculline, picrotoxin, penicillin, quinolinic acid and strychnine. It potentiates the anticonvulsant activity of phenobarbital without potentiating its hypnotic activity. Apparently, it has little or no cardiovascular, antispasmodic, or tranquilizing activity. Its therapeutic index (LD_{50}/ED_{50}) is very favorable, the lethal dose is 4 to 8 times greater than that of the anticonvulsant dose (Swinyard and Woodhead, 1982; Penry and Dean, 1993).

1.2 Mechanisms of Action

Antiepileptic drugs can be divided mechanistically into at least three classes based on the ability to block sustained repetitive firing (SRF) by enhancing

GABAergic inhibition or by increasing sodium-channel inactivation, and finally inhibiting pacemaker-driven, repetitive firing by blocking T-calcium current (Macdonald, 1989).

The mechanisms of action of sodium valproate are far from clear. It has been suggested that valproate acts in multiple sites by several mechanisms (Penry, 1988).

1.2.1 Effects on GABA system

Although the exact mechanism of action of valproate is unknown, several theories have been proposed. One possible theory involves valproate-induced changes in the metabolism of GABA and enhanced GABA activity within the brain, causing increased brain levels of GABA and improved neuronal responsiveness to GABA. Those changes appear to be associated with seizure control. Other researchers have postulated that valproate exerts its antiepileptic activity by direct neuronal effects (Penry and Dean, 1993). In some certain brain regions, VPA enhances brain GABA concentration and may enhance release of GABA from nerve terminals (Johnston, 1984; Macdonal, 1989). Valproic acid has also been demonstrated to exhibit an activation of GAD (Phillips and Fowler, 1982) and a strong inhibition of SSADH, an enzyme in the GABA degradation pathway (Zeise, Lasparow, and Zeiglgansberger, 1991). Subsequent research suggested that VPA selectively enhanced post-synaptic GABA responses, although the clinical evidence of this effect was not known (Davies and Richens, 1993; Davis et al, 1994). Administration of VPA to experimental animals causes an increase in whole brain and synaptosomal GABA. In mouse synaptosomes, VPA 125 mg/kg was found to increase GABA levels via inhibition of GABA-T (Loscher and Vetter, 1985).

1.2.2 Effects on amino acid neurotransmitters other than GABA

There are evidences suggesting that valproic acid reduces neuron transmission mediated by GHB (inhibition of aldehyde reductase) as well as those mediated by excitatory amino acids such as aspartic acid, glutamic acid (Johnston, 1984). VPA increases extracellular 5-hydroxy tryptamine (5-HT) concentration and may therefore be expected to enhance serotonergic transmission (Whitton and Fowler, 1991).

1.2.3 Effects on membrane ion channels

VPA also appears to reduce sustained repetitive firing (SRF) as do phenytoin and carbamazepine. At low concentrations VPA diminished SRF of sodium-dependent action potentials in mouse spinal cord and cortical neuron culture (McLean and Macdonald, 1986). VPA has been noted to protect mice against convulsions produced by potassium channel blockers (Moore et al., 1984). The relevance of these actions on neuronal potassium and sodium conductance to clinically important anticonvulsant mechanisms is unclear (Davis et al.,1994). However VPA did not alter T-calcium current (Macdonald, 1989).

2. Pharmacokinetics

Sodium valproate has been shown to be completely absorbed (>95%) following oral administration, with peak plasma concentration of valproic acid occurring at 1 to 4 hours after ingestion of the plain tablets (Richens and Perucca , 1993). The distribution of valproic acid is largely restricted to the extracellular water. Values for the apparent volume of distribution have been reported as 0.1-0.4 l/kg. The concentration of VPA in plasma that appears to be associated with therapeutic effects

is approximately 30 to 100 μg/ml Concentrations in brain and cerebrospinal fluid are much lower than plasma levels and appear to be related to the free drug concentration in plasma. In human, the brain concentration has been shown to be 6.8-27.9% of the plasma concentration. Valproic acid is highly bound (approximately 90%) and binding is reduced by free fatty acids as well as in patients with hypoalbuminaemia, liver and renal disease. Because of the drug's low pKa (4.95), it is secreted into saliva in small amounts which do not reflect the plasma concentration. Valproic acid is almost completely metabolized prior to excretion, only 1-3% of the ingested dose being excreted unchanged in the urine. The major elimination pathway is via conjugation with glucuronic acid (20-70%) (Rall and Schleifer, 1990; Richens and Perucca, 1993). At least five main metabolic pathways for VPA have been described in humans: glucuronidation, β-oxidation, ω- ω_1- and ω_2 oxidation (Zaccara et al, 1988). The *E* (trans.) isomer of 2-n-propyl-2-pentanoate (2-ene-valproate; $\Delta^{2(E)}$-VPA) is the major active metabolite of the antiepileptic drug, valproate, in various species, including humans. The compound was slightly more active than VPA against seizure induced by PTZ in mice and seems to lack the teratogenic and possibly also the hepatotoxic adverse effects of VPA (Loscher, 1992).

The plasma half-life varies between 9-16 hours, depending on concomitantly administered drugs and metabolic variation, patients receiving concomitant hepatic enzyme-inducing antiepileptic drug exhibiting half-life values in the lower end of this range. The elimination half-life of valproate ranges from 10 to 16 hours when given as monotherapy, and 8 to 9 hours when valproate is administered with other antiepileptic drugs (Penry and Dean, 1993). Plasma clearance of valproic acid ranges from 0.4 to 0.6 l/h in healthy volunteers are independent of hepatic blood flow, decreases with increasing pediatric age (Davis, et al., 1994).

3. Adverse Drug Reactions

3.1 Common side effects

Valproate therapy tends to cause gastrointestinal effects (anorexia, indigestion, heartburn, and nausea) rather than neurological effects (Davis et al., 1994). The incidence of these gastrointestinal disturbances can be reduced considerably by using the enteric-coated dosage form. Weight gain, temporary hair thinning or loss which occasionally may be decreased by dosage reduction. Currently, however, no specific relationship has been found between valproate serum concentrations and hair loss or growth (Penry and Dean, 1993). VPA therapy is generally associated with fewer neurological effects than other antiepileptic drugs. Additionally, It has minimal impairment on cognitive function and behavior compared with phenytoin or phenobarbital. The incidence of adverse effects has been suggested to increase at plasma concentrations higher than 120 μg/ml (Schmidt, 1982).

3.2 Serious adverse reactions

This drug offers several significant advantages but has the disadvantage of occasionally being associated with severe and sometimes lethal hepatotoxicity. To 1988 at least 112 deaths from such hepatotoxicity were known worldwide . Most instances of hepatotoxicity have occurred in infants or young children (Eadie et al., 1990), though, it is a rare, increasing idiosyncratic, and non dose-related adverse reaction. The risk declined with age and was very low in monotherapy patients (1 per 37,000) (Dreifuss et al., 1987). Several possible biochemical mechanisms responsible for the hepatotoxicity have been proposed, but

the matter has not been decided. However, certain evidence suggests that the drug's δ-dehydrogenation derivative 4-en-valproic acid is hepatotoxic (Eadie et al., 1990).

Pancreatitis and hematologic side effects such as thrombocytopenia are rarely profound. The underlying mechanism of idiosyncratic reaction that has been reported with the use of valproate is unknown. The reaction generally occurs within the first 6 months of therapy and can be fatal (Blaise and Bourgeois, 1988)

Sodium valproate has been shown to be teratogenic in animal and reports of abnormalities in the offspring of epileptic mothers taking sodium valproate have been published. Spina bifida is the most common abnormality and occurs in about 1% of newborn from mothers taking valproate (Richen and Peruca,1993). An increased risk of neural tube defects has been observed when valproate is administered during the first trimester of pregnancy. The overall risk of various neural tube defects is about 1% to 2% (Penry and Dean, 1989).

Valproate overdosage can result in deep coma. Death from valproate overdosage has been reported with plasma concentrations of 1,970 μg/ml (Schmidt, 1982).

4. Drug interactions

Valproate is often administered with other antiepileptic drugs that can lead to clinically significant pharmacologic interactions. Little is known about its interaction with drugs other than antiepileptics, except for the increase in the half-life of antipyrine (Furlanut, Schiaulini and Rizzoni, 1982). Side effects of valproate were seen in about one-fourth of patients treated with other enzyme-inducing antiepileptic

drugs such as carbamazepine, phenobarbital, primidone, or phenytoin which markedly accelerate the metabolic conversion of valproate, particularly in children (Schmidt, 1982). The sedative side effects often seem to be due to a rise in the phenobarbital plasma concentration when valproate is added to phenobarbital because of the inhibition of metabolism of phenobarbital by valproic acid resulting in a prolonged elimination half-life. Valproate does not induce the metabolism of contraceptive hormones in birth control pills. As a result, valproate has not been associated with oral contraceptive failure and is an excellent antiepileptic drug for women who want to be assured of maximum contraceptive protection (Blaise and Bourgeois ,1988; Penry and Dean, 1989).

(N-hydroxymethyl)-2-propylpentamide (HMV)

(N-hydroxymethyl)-2-propylpentamide (HMV) is a new valproate derivative which was synthesized by Assistant professor Dr. Chamnan Patarapanich. The HMV consists of two part, one is hydroxymethylamide group and another is valproic acid (Figure 2)

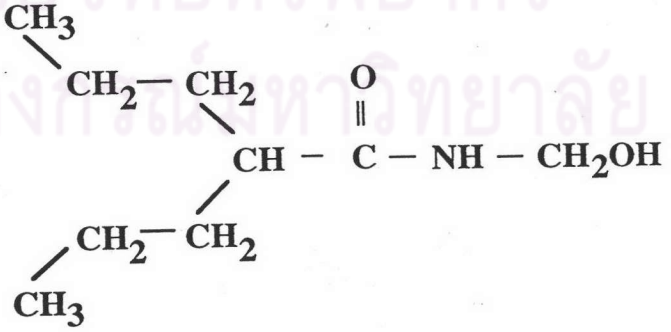

Figure 2 The structure of (N-hydroxymethyl)-2-propylpentamide (HMV)