2.1 Classification of seizures and epilepsies

The international League Against Epilepsy (ILAE) has proposed two major schemes for classification of seizures and epilepsies. The first one is the International Classification of Epileptic Seizures (Commission on Classification and Terminology of the ILAE, 1981) (Table 2.1) that based on the clinical manifestations of the attacks and the pattern of the EEG. The second one is the International Classification of Epilepsies and Epilepsy Syndromes (Commission on Classification and Terminology of the ILAE, 1989) (Table 2.2) that based on the presumed site of origin of the seizures (localization–related or generalizes) and the etiology of the seizures (idiopathic or cryptogenic or symptomatic).

Epileptic seizures are generally classified into focal (partial) and generalized. The clinical manifestation of focal seizures varies depending upon the origin of epileptic discharges (the epileptic focus), and includes motor, sensory, autonomic, and psychic symptoms. Seizures are defined as simple partial if there is no loss of consciousness, and complex partial if there is a loss of consciousness. When the seizure discharge becomes sufficiently widespread (or generalized), and includes a strong participation of motor system circuitry, the result is a convulsive response that typically includes both tonic (sustained contractions) and clonic (oscillating contractions and relaxations) components. Absence seizures are primary generalized seizures that do not include strong motor system recruitment. Such seizures appear to be nearly immediately generalized. Even focal seizures, however can become generalized (secondary generalized seizures) (Commission on Classification and Terminology of the ILAE, 1981; Commission on Classification and Terminology of the ILAE, 1989).

Table 2.1 International classification of epileptic seizures
(modified from Commission on Classification and Terminology of the ILAE, 1981)

I Partial seizures (seizures begin locally)

A. Simple (without impairment of consciousness)
   1. with motor symptoms
   2. with special sensory or somatosensory symptoms
   3. with psychic symptoms

B. Complex (with impairment of consciousness)
   1. simple partial onset followed by impairment of consciousness-with or without automatisms
   2. impaired consciousness at onset-with or without automatisms

C. Secondarily generalized (partial onset evolving to generalized tonic-clonic seizures)

II Generalized seizures (bilaterally symmetrical and without local onset)

A. Absence
B. Myoclonic
C. Clonic
D. Tonic
E. Tonic-clonic
F. Atonic
G. Infantile spasms

III Unclassified seizures

IV Status epilepticus

Table 2.2 International classification of epilepsies and epilepsy syndromes (modified from Commission on Classification and Terminology of the ILAE, 1989)

I Localization-related (focal, partial) epilepsies and syndromes

- A. Idiopathic (with age-related onset)
  1. Benign childhood epilepsy with centro-temporal spike
  2. Childhood epilepsy with occipital paroxysms
  3. Primary regarding epilepsy
- B. Symptomatic
  1. Chronic progressive epilepsia partialis continua of childhood
  2. Syndromes characterized by seizures with specific modes of precipitation
- C. Cryptogenic

II Generalized epilepsies and syndromes

- A. Idiopathic
  1. Benign neonatal familial convulsions
  2. Benign neonatal convulsions
  3. Benign myoclonic epilepsy in infancy
  4. Childhood absence epilepsy (pyknolepsy)
  5. Juvenile absence epilepsy
  6. Juvenile myoclonic epilepsy
  7. Epilepsy with grand mal (GTCS) seizures on awakening
  8. Other generalized idiopathic epilepsies not defined above
  9. Epilepsies with seizures precipitated by specific modes of activation
- B. Cryptogenic or symptomatic
  1. West syndrome
  2. Lennox-Gastaut syndrome
  3. Epilepsy with myoclonic-astatic
  4. Epilepsy with myoclonic absence

Table 2.2 (continue) International classification of epilepsies and epilepsy syndromes

C. Symptomatic
    1. Nonspecific etiology
        a. Early myoclonic encephalopathy
        b. Early infantile epileptic encephalopathy
        c. Other symptomatic generalized epilepsies not defined above
    2. Specific syndromes

III Epilepsies and syndromes undetermined whether focal or generalized

A. With both generalized and focal seizures
    1. Neonatal seizures
    2. Severe myoclonic epilepsy in infancy
    3. Epilepsy with continuous spike-waves during slow wave sleep
    4. Acquired epileptic aphasia
    5. Other undetermined epilepsies not defined above
B. Without unequivocal generalized of focal features

IV Special syndromes

A. Situation-related seizures
    1. Febrile convulsions
    2. Isolated seizures or isolated status epilepticus
    3. Seizure occurring only when there is an acute metabolic or toxic event

2.2 Causes of epilepsy

Although a significant number of epilepsy cases are idiopathic, it is estimated that up to 50% of epilepsy cases are associated with a previous neurological insult and are called acquired epilepsy (DeLorenzo, 1991). The other 50% of epilepsy cases occur in the absence of other brain abnormalities. These epilepsies are called idiopathic, in that there is no known cause for the manifestation of epilepsy. Ongoing research in the field of medical genetics has led to the recent elucidation of an underlying cause for some of these idiopathic cases with the identification of cell migration abnormalities (Rakic, 2000; Lee et al., 2001; Sato et al., 2003) and numerous gene mutations in humans (Bertrand et al., 1998; Biervert et al., 1998; Wallace et al., 1998) and mouse models of epilepsy (Puranam and McNamara, 1999) that may underlie some of these idiopathic epilepsies. Recently, monogenic mutations of ion channel genes have been found in patients with inherited epilepsies. These epilepsy mutations have been identified in multiple voltage- and ligand-gated ion channels including the sodium, calcium and potassium voltage-gated ion channels and nicotinic cholinergic and $GABA_A$ receptor ligand-gated ion channel (Hirose et al., 2002; Mulley et al., 2003). However, in the majority of idiopathic cases, the underlying cause of the epileptic phenotype is still not known.

In the remaining half of epilepsy cases, a known cause or injury produces a permanent plasticity change in a previously normal brain leading to the development of acquired epilepsy. This transformation of healthy central nervous system (CNS) tissue with a functional balance between excitation and inhibition to brain tissue having a hyperexcitable neuronal population of neurons is called epileptogenesis (Lothman, Bertram, and Stringer, 1991; McNamara, 1999).

2.3 Epileptogenesis

There is much knowledge on the molecular cellular and electrophysiologic mechanisms of epileptogenesis because of the availability of resected focal epileptic tissue from patients with medically intractable focal epilepsy and the development and characterization of animal models for various types of epilepsies. At the single-cell or local-circuitry levels, *in vitro* models of hyperexcitability or synchronization have provided

additional insight into the various mechanisms of epileptogenesis. Multiple factors contribute to epileptogenesis, such as intracellular, intrinsic membrane, and extracellular mechanisms. Based on animal data, three key elements contribute to the development of the hyperexcitability needed for epileptogenesis: 1) the capability of membranes in pacemaker neurons to develop intrinsic burst discharges; 2) the reduction of GABAergic inhibition; and 3) enhancement of synaptic excitation through recurrent excitatory circuits (e.g., mossy fiber sprouting in hippocampal sclerosis). Although intrinsic membrane hyperexcitability provides a substrate for epileptogenesis, circuit dynamics are more important for paroxysmal electrophysiologic tendencies. Multicellular synchronization is necessary for the EEG and behavioral seizure expression and is critical for the expression of interictal and ictal activities and the generation of cellular paroxysmal depolarization shifts. Although hyperexcitability may be easily reconciled with changes in neuronal circuitry, synchronization may involve other cell types (e.g. glia) and changes in the extracellular space (Najm, Ying, and Janigro, 2001; Avanzini and Franceschetti, 2003).

2.3.1 Excitability changes leading to epileptogenesis

The neuronal cytoplasmic membrane consists of a lipid bilayer that is largely impermeable to ions. However, ions can be actively transported across the membrane by ionic pumps and can move through voltage-gated or ligand-gated channels depending on their electrochemical gradients. Transient changes in membrane potential are generated by transmembrane ionic movements through voltage-gated and ligand-gated channels that are selectively permeable to specific ions. Membrane depolarizations activate voltage-gated sodium channels, which cause the rising phase of the action potential. Subsequently, various voltage-gated potassium channels, which are involved in the repolarising phase, are activated. Immediately after opening, sodium channels enter an inactivated state in which the channel pore is blocked by a ball-and-chain mechanism that prevents further flow of sodium ions. At the peak of the action potential the majority of sodium channels are inactivated. However, the few channels that fail to inactivate carry the persistent fraction of the sodium current; although small, this current can be sufficient to drive the membrane towards the firing threshold. This mechanism underlies the bursting properties of the "intrinsically bursting" subpopulation of pyramidal neurons in layer V. (Franceschetti et al.,

1995; Mantegazza, Franceschetti, and Avanzini, 1998; Avanzini and Franceschetti, 2003). As a result of widespread expansion of their axonal arborisation, intrinsically bursting neurons establish synaptic contact with a large number of neighbouring neurons and sustain highly synchronised activities (Chagnac-Amitai and Connors, 1989).

The observation that neurons in an epileptic neuronal aggregate consistently discharge in the form of protracted bursts of action potentials has been central to the investigation of epileptogenic cell mechanisms. These bursts—called paroxysmal depolarization shifts—were initially described by Matsumoto and Ajmone-Marsan (1964), who observed them in penicillin-induced cortical foci. In normal brain, a similar phasic type of cell discharge is seen in subpopulations of intrinsically bursting neurons of the neocortex and area 3 of Ammon's horn (CA3) in the hippocampus. In both of these cortical structures, intrinsically bursting neurons are particularly involved in local synchronization. Spontaneous or stimulus-evoked paroxysmal depolarization shifts frequently occur in physiologically non-bursting neurons in both induced epileptogenic foci and in epileptic human tissue removed during surgery. This finding shows that, in focal epilepsies at least, paroxysmal depolarization shifts are a reliable marker of an established epileptogenic condition (Prince, 1985; Avanzini and Franceschetti, 2003).

The relevance of these mechanisms to human and experimental epilepsy are discussed in relation to the different types of voltage-gated and ligand-gated channels.

A pathophysiological role of sodium channels in human epilepsies was originally suggested on the basis of indirect observations, such as the inhibitory effect of clinically effective antiepileptic drugs on sodium currents (Lipicky, Gilbert, and Stillman, 1972; Ragsdale et al., 1996). Studies of surgically resected human tissue have shown that changes in the ratio between different sodium-channel subtypes are associated with drug-refractory seizures in TLE (Lombardo et al., 1996). Several groups have reported genetically determined changes in the molecular structure of sodium channels in patients with familial generalized epilepsies with febrile seizures ($GEFS^{+}$) and in those with severe myoclonic epilepsy of infancy (Escayg et al., 2000).

Calcium currents, which are defined according to their activation threshold and kinetics, underlie both transient and sustained cell-membrane depolarizations. By use of blockers selective for different calcium currents, Beck and colleagues (1998) found upregulation of calcium-current density in hippocampal epileptogenesis, both in patients with epilepsy and in rats exposed to kainic acid. Moreover, Straub and co-workers (2000) have shown that a high-threshold calcium channel is implicated in epileptogenesis *in vitro*; this channel has a complex dose dependence and a differential effect in hippocampal and neocortical structures.

The pathogenetic role of a genetically determined the M-current, one of the many types of potassium current, defect has been found in benign neonatal familial convulsions (Biervert et al., 1998). Two potassium-channel subunits (KCNQ2 and KCNQ3) contribute to the M current, and mutations of either of the genes encoding these subunits (located at the 20q13 and 8q24 chromosomal loci, respectively) lead to a M-current impairment associated with the phenotype of benign neonatal familial convulsions.

In 1995, Phillips and coworkers studied 27 individuals from a large Australian family affected by autosomal dominant nocturnal frontal-lobe epilepsy. Mutations in the neuronal nicotinic acetylcholine receptor $\alpha 4$ and $\beta 2$ subunit genes have been detected in families with autosomal dominant nocturnal frontal lobe epilepsy. Both receptors are components of neuronal acetylcholine receptor, a ligand-gated ion channel in the brain (Steinlein et al.,1995; Gambardella et al., 2000).

Mutations in the $GABA_A$ receptor $\gamma 2$ subunit genes was found as a cause of $GEFS^+$. $GABA_A$ receptor and their auxiliaries may be involved in the pathogenesis of this subtype and even in simple febrile convulsions (Hirose et al., 2002).

2.3.2 Amino acid neurotransmitter system in epilepsy

The long history of the involvement of various neurotransmitter systems (particularly excitatory and inhibitory amino acids) in the generation of sustained local epileptic hyperactivity (epileptic foci), as well as the initiation and spread of seizures is well documented (Meldrum, 1989; Bardford, 1995). It is generally known that glutamate and

GABA are major excitatory and inhibitory neurotransmitter of the CNS, respectively (Kaura et al., 1995). An imbalance between excitation and inhibition produced by a decrease in GABAergic and/or an increase in glutamatergic transmission has been associated with the generation of epileptic pathological condition, both in animal models and in humans (Bardford, 1995; Pena and Tapia, 2000).

2.3.2.1 Excitatory amino acid neurotransmitters in epilepsy

Of the excitatory amino acids (EAAs), glutamate is postulated to be the major excitatory neurotransmitter in the mammalian CNS. Other EAA neurotransmitters include aspartate, homocysteine sulphinic acid, cysteine sulphinic acid, homocysteic acid and quinolinate (Trist, 2000). A disorder in glutamate-mediated excitatory neurotransmission has long been a candidate as a central factor in the etiology of at least some forms of human and experimental epilepsy (Bardford, 1995).

Glutamate is synthesized in the cytoplasm and stored in synaptic vesicles by an uptake system that depends on the proton electrochemical gradient, the vesicular glutamate transporters (VGLUTs). Following its exocytotic release, glutamate activates ionotropic glutamate receptors for fast excitatory neurotransmission and metabotropic receptors for slower modulatory affects on transmission. There are three main types of ionotropic glutamate receptors: *N*-methyl-*D*-aspartate (NMDA), kainic acid and α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA). To terminate the action of glutamate and maintain its extracellular concentration below excitotoxic levels, Na^+-dependent high affinity glutamate transporters (excitatory amino acid transporters: EAATs) located on the plasma membrane of neurons and glial cells rapidly remove glutamate from the extracellular space (Seal and Amara, 1999; Danbolt, 2001; Balcar, 2002; Shigeri et al, 2004). Glutamate is thought to be released not only synaptically but also extrasynaptically by exocytosis (Bezzi et al., 2001), cystine-glutamate antiporter (Baker, 2002) and volume-regulated anion channels (Haskew, Mongin, and Kimelberg, 2002). However, most of the glutamate is released synaptically and transits through the glutamate–glutamine cycle before being repackaged into synaptic vesicles (Hamberger et al., 1979). Glutamate taken up into glial cells is metabolized to glutamine, which is then transported back into neurons,

converted to glutamate and sequestered into synaptic vesicles by the VGLUTs (Fig. 2.1) (Shigeri et al, 2004) .

In both human temporal lobe epilepsy (During and Spencer, 1993) and the kainate model (Wilson et al., 1996), extracellular concentrations of glutamate markedly increase in the epileptogenic hippocampus during spontaneous seizures. In the kindling model too, several microdialysis studies revealed a significant glutamate increase during kindled seizures in both the hippocampus (Zhang et al., 1991; Minamoto et al., 1992; Ueda and Tsuru, 1995) and the amygdala (Kaura et al., 1995). Furthermore, a pronounced glutamate release followed by activation of ionotropic and/or metabotropic receptors have been associated with glutamate excitotoxicity and neuronal death in several structures during status epilepticus (Millan et al., 1993; Oxbury and Whitty, 1971). In human epileptic study, Perry and Hansen (1981) reported a rise in glutamate levels and no change in aspartate and GABA levels. Elevated cerebrospinal fluid glutamate and aspartate in untreated patients were reported by Plum (1974) and Logothetis and Bovis (1984). Studies of neuroactive amino acids in surgically excised focally epileptic human brain tissue depict the involvement of these amino acids in epilepsy, in that concentrations of glutamate, aspartate and glycine are significantly increased in epileptogenic cerebral cortex (Sherwin, 1999).

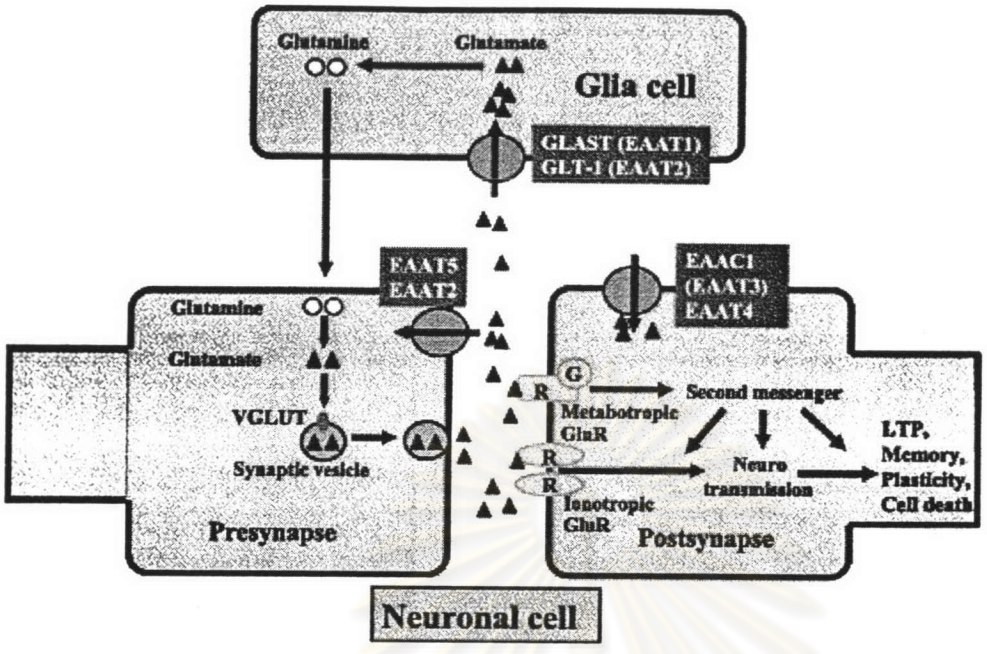


Figure 2.1 Mechanism of excitatory neurotransmission in the mammalian central nervous system (Shigeri et al, 2004).

2.3.2.2 Changes in glutamate transporters

The glutamate transporter powerfully regulates extracellular glutamate in the synaptic cleft via Na^+-coupled reuptake mechanisms (O'Shea, 2002). Studies with antisense knockdown of specific transporter subtypes have revealed that the astrocytic glutamate transporters, EAAT1 (known as glutamate transporter (GLAST) in rodents) and EAAT2 (known as GLT1 in rodents), are responsible for removing the majority of the extracellular glutamate released during neurotransmission, whereas the glutamate transporter expressed on neurons, EAAT3 (EAAC1 in rodents), may play a more subtle role in regulating excitability and signaling (Amara and Fontana, 2002). Impairment of this system, therefore, would be expected to contribute to excessive glutamate levels, chronic epileptogenesis, and neurodegeneration. In fact, GLT1-deficient mice developed early onset epilepsy (Tanaka et al., 1997), suggesting that GLT1 is important for normal synaptogenesis and seizure inhibition in neonatal animals (Maragakis and Rothstein, 2001). In mice deficient in GLAST, the duration of generalized kindled-seizures was significantly prolonged, and sensitivity to PTZ was increased, compared with wild type mice (Watanabe et al., 1999). In human TLE with hippocampal sclerosis, there is increased EAAC1 immunoreactivity on the remaining granule and pyramidal cells, decreased GLT1 in the hilus and CA1 (associated with neuronal loss), and an increase in GLAST in the CA2/3 (Mathern et al., 1999).

2.3.2.3 GABA release and uptake

GABA, the principal inhibitory neurotransmitter in the cerebral cortex, maintains the inhibitory tone that counterbalances neuronal excitation. When this balance is perturbed, seizures may ensue. GABA is formed within GABAergic axon terminals by transamination of α-ketoglutarate to glutamic acid, which is then decarboxylated by glutamic acid decarboxylase (GAD) to GABA. It is released into the synapse and then acts at GABA receptors (Fig. 2.2) (Treiman, 2001).

The two main types of ionotropic GABA receptors ($GABA_A$ and $GABA_B$) are coupled to chloride and potassium ionophores, respectively. A third receptor subtype, $GABA_C$, has also been reported but its physiological function is unclear at present

(Avanzini and Franceschetti, 2003). After release from the presynaptic axon terminals, GABA is rapidly removed by uptake into both glia and presynaptic nerve terminals and then is catabolized by GABA transaminase to succinic semialdehyde. Succinic semialdehyde is converted to succinic acid by succinic acid semialdehyde dehydrogenase and then enters the Krebs cycle (Treiman, 2001).

Hippocampal microdialysis studies demonstrated what appeared to be an insufficient elevation of extracellular concentrations of GABA during kindled seizures. During these seizures, GABA was elevated approximately 250–350% from the baseline level, compared with a 550–650% increase in glutamate levels (Minamoto et al., 1992; Ueda and Tsuru, 1995). These increases were comparable to those measured in human TLE (During and Spencer, 1993). In the kindled amygdala, extracellular glutamate was increased by 200–300% during kindled seizures, while GABA was reduced by 67% (Kaura et al., 1995).

Chaudieu and coworkers (1987) reported that kindling enhanced presynaptic GABA transport. Hirao and colleagues (1998) also demonstrated increased expression of mRNAs for GABA transporters GAT1 (located on presynaptic terminals of GABAergic neurons) in the hippocampus and GAT3 (located on astrocytes) in the limbic forebrain. Furthermore, selective inhibitors of GABA transporters, including SKF89976A, tiagabine and NNC771, have potent antiepileptic effects on amygdala- and hippocampal-kindled seizures (Morimoto et al., 1997). Recently, it has been shown that GAT1 immunoreactive neurons are reduced after 90–100 kindled seizures in association with the emergence of spontaneous seizures (Sayin et al., 2003).

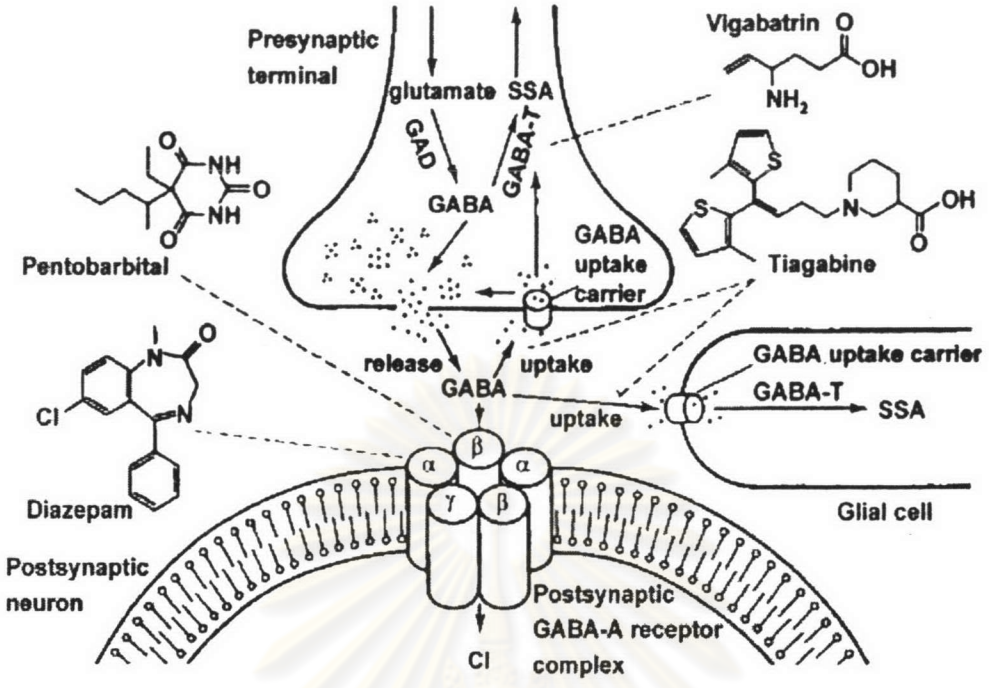


Figure 2.2 GABAergic synapse. GABA is synthesized in the presynaptic terminal. After release it causes the $GABA_A$ receptor on the postsynaptic neuron to increase inward chloride conductance, as do many other drugs described in the text, including the barbiturates and benzodiazepines. Synaptic GABA is taken back up into the presynaptic terminal and into glial cells. Reuptake inhibitors, such as tiagabine, and drugs that block GABA metabolism, such as vigabatrin, thus increase synaptic GABA levels. SSA, succinic semialdehyde; GAD, glutamic acid decarboxylase; GABA-T, GABA transaminase (Treiman, 2001).

2.3.2.4 Glycine

Glycine exerts multiple functions in the central nervous system, as an inhibitory neurotransmitter through activation of specific, Cl^--permeable, ligand-gated ionotropic receptors and as an obligatory co-agonist with glutamate on the activation of NMDA receptors (Aragón and López-Corcuera, 2003).

Glycine is one of the major inhibitory neurotransmitters in posterior areas of the vertebrate CNS. In the spinal cord and brain stem, glycinergic interneurones provide an inhibitory feedback mechanism that controls the motor rhythm generation during movement and they also play an important role in the coordination of spinal reflex activity (Grillner, et al., 1998; Legendre, 2001). The glycine-mediated neurotransmission involves storage of the transmitter in synaptic vesicles (Dumoulin et al., 1999), transmitter release following neuron depolarization (Mulder and Snyder, 1974) and glycine binding to, and activation of, specific, Cl^- permeable, ligand-gated ionotropic receptors on the postsynaptic neuron (Fig. 2.3) (Aragón and López-Corcuera, 2003). The activation of receptor generates inhibitory postsynaptic potentials as a result of increasing Cl^- conductance that are antagonized by strychnine (Werman, Davidoff, and Aprison, 1967).

In contrast to its inhibitory action, glycine exerts a positive modulation on excitatory glutamatergic neurotransmission through NMDA receptors (Fig. 2.4) (Johnson and Ascher, 1987). The glycine was shown to be a co-agonist implying that both NMDA and glycine must be present on the receptor before the Mg^{++} block is overcome and the channel opens (Kleckner and Dingledine, 1988). In infant brains, the amount of binding site to the glycine was higher in temporal cortex and hippocampus than in basal ganglia and was also higher than in comparable areas of adult brain (D'Souza et al., 1993). The NMDA receptor for glycine can be distinguished from the inhibitory glycine receptor by the fact that it is strychnine-insensitive. The binding site for glycine has been identified to be on the NR1 subunit (Williams et al., 1996), whereas the recognition site for glutamate and NMDA has been shown to be on the NR2 subunits (Laube et al., 1997).

Glycine and GABA seem to share a common vesicular inhibitory amino acid transporter (VIAAT) as suggested by its localization to synaptic vesicles in

glycinergic terminals (Aragón and López-Corcuera, 2003). Two glycine transporter (GLYT) proteins, GLYT-1 and GLYT-2, are members of the large family of Na^+/Cl^--dependent neurotransmitter transporters. Both GLYT1 and GLYT2 are expressed in the spinal cord and brain stem, a location consistent with their role in terminating glycinergic transmission. However, GLYT-1 is also expressed in several regions of the forebrain that are devoid of glycinergic neurotransmission. Thus, GLYT-1 may regulate NMDA receptor function in these areas by controlling the levels of extracellular glycine available to allosterically modulate the activity of these receptors (Cooper, Bloom, and Roth, 2003).

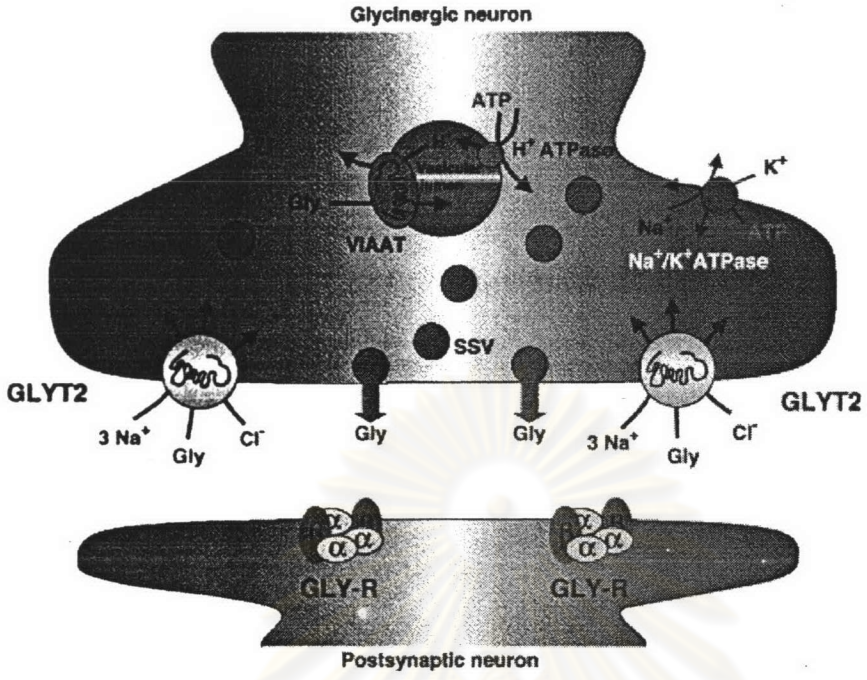


Figure 2.3 Schematic representation of a glycinergic inhibitory synapse. VIAAT: vesicular inhibitory amino acid transporter; SSV: small synaptic vesicles; GLYT2: glycine transporter two; GLY-R: strychnine-sensitive glycine receptor (Aragón and López-Corcuera, 2003).

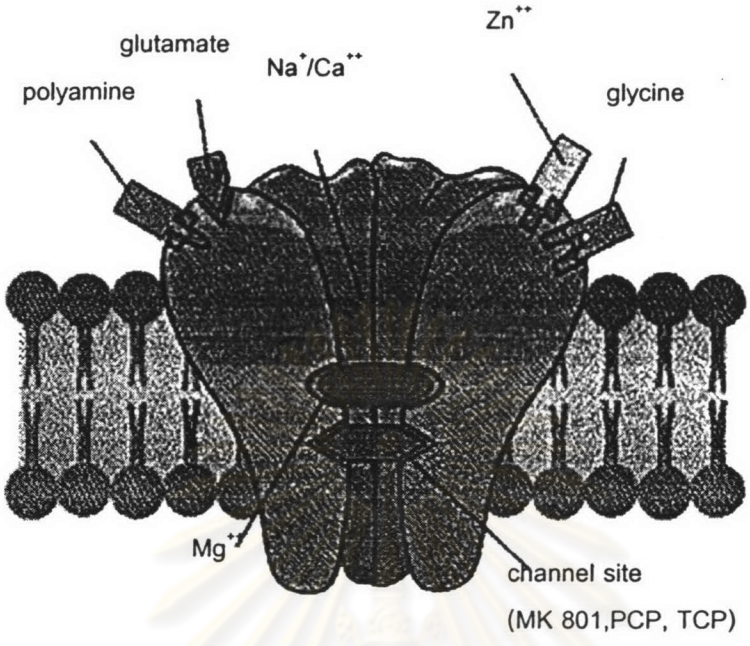


Figure 2.4 Schematic illustration of NMDA receptors and binding sites. The NMDA receptor complex is a large protein assemblage that has multiple binding sites for different ligands, including an NMDA binding site, a strychnine-insensitive glycine binding site, and a binding site within the channel for certain noncompetitive antagonists; each of which can bind several different compounds. Within the channel, non-competitive antagonists, such as (+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d] cyclohept-5,10-imine maleate (MK801), ketamine, phencyclidine (PCP) and 1-(1-thienyl-cyclohexyl)piperidine (TCP); can bind.

(http://homepage.psy.utexas.edu/HomePage/Class/Psy301/Salinas/sec2/L&M/23.GIF)

The concentrations of glutamate, aspartate and glycine are significantly increased in surgically excised focally epileptic human tissue in cerebral cortex (Sherwin, 1999). A glycine site antagonist with high selectivity and affinity was shown to be anticonvulsant after intravenous (i.v.) administration in NMDA-induced convulsions (Chapman and Dickenson, 1995). In addition, felbamate, remacemide and riluzole are anticonvulsant medications which—at least in part—convey their effect via antagonism at the glycine$_B$ site (McCabe et al., 1993).

2.4 Seizure-induced neuronal injury

The association of epilepsy with neuronal damage was recognized in the 19th century by neuropathologists who observed hippocampal damage in autopsy studies of people with epilepsy. Among the events that occur in response to the initial insult, neuronal death has received significant attention as the propagating factor that links the initial insult with the epileptic condition. However, to date the role of neuronal death in the development of epilepsy remains controversial. Accumulating evidence suggests that neuronal cell death may be both a cause and a consequence of epileptic seizures. The evidence that seizures cause brain injury comes from the demonstration that intense seizure activity associated with SE can cause hippocampal damage in part by excessive activation of glutamate receptors and resultant excitotoxicity (Slovitor and Dempster, 1985; Meldrum and Chapman, 1999; and Patel, 2004). The idea that neuronal death can cause epilepsy is supported by the fact that surgical removal of a damaged hippocampus improves the condition of epilepsy patients (Bruton, Stevens, and Frith, 1994).

Seizure activity results in a large number of changes and cascades of events at a cellular level. Changes in gene expression, receptor composition, synaptic physiology and the activation of some late cell death pathways have been reported (Cook, 2002). It has long been known from both human studies and experimental models, that prolonged seizure, and particularly SE, are associated with neuronal injury. Following an episode of SE, acute neuronal necrosis is observed in vulnerable regions which include the pyramidal cells of hippocampus and cerebral cortex, the thalamus, and the Purkinje cells of the cerebellar cortex (Ben-Ari, 1985; Turski et al., 1989; Men et al., 2000; and Thom, 2004). Although

seizures can induce changes in multiple areas of the brain, the hippocampus has been particularly well studied because this region is the most vulnerable to seizure-induced injury (Holmes, 2002). The mechanisms of reversible and irreversible neuronal injury in epilepsy include: excitotoxic injury through the activation of glutamate receptors (NMDA and also AMPA receptors); the influx of Ca^{++}; the activation of cell-stress and apoptotic pathways; and mitochondrial dysfunction has been reported. It is likely that additional factors, including age at onset of seizures, the type of seizure and genetic factors may influence the susceptibility to epilepsy-mediated neuronal injury in any individual (Sutula, Hagen, and Pitkanen 2003; Thom, 2004).

2.4.1 Oxidative Stress

Reactive oxygen species (ROS) are a part of normal human metabolism. ROS has been implicated in a variety of acute and chronic neurologic conditions (Bonfoco et al., 1995) including convulsions (Bruce and Baudry, 1995; Ueda et al., 1997). Whereas a precise role for ROS in the epilepsies remains to be defined, a general role for ROS in seizure-induced neuronal death is supported in part by the observations that repeated seizures result in increased oxidation of cellular macromolecules (Liang et al., 2000; Patel, Liang, and Roberts, 2001; Patel, 2004).

Cell damage is believed to involve excitotoxicity, whereby excessive glutamate causes over activation of the NMDA receptors, with a resultant accumulation of intracellular calcium. A high level of intracellular calcium leads to generation of ROS. When being produced in excess, ROS can cause tissue injury including lipid peroxidation, DNA damage, and enzyme inactivation (Holmes, 2002). A number of studies suggest that oxidative stress, defined by excessive production of free radicals, may play an important role in the etiology of seizure-induced neuronal death. As reported by Ueda et al. (1997), seizures induced by kainic acid (KA) were able to produce lipid radicals in the hippocampus of rats leading to neuronal damage. In addition, Dal-Pizzol and coworkers (2000) demonstrated that there was a slight enhancement in the level of thiobarbituric acid reactive substances measured 12-14 h after the end of status epilepticus induced by pilocarpine and kainic acid in hippocampus. The superoxide dismutase activity decreased

which was associated with high hydroperoxide concentration after long-lasting SE period induced by pilocarpine (Bellissimo et al., 2001).

2.4.2 Mitochondrial dysfunction

Mitochondrial oxidative phosphorylation is the primary source of energy for neuronal metabolism. Mitochondrial function is a crucial determinant of cell death and oxidative stress, thus it acts as the "stress sensor", and in extreme circumstances "executioner" of the cell (Green and Reed, 1988). Mitochondrial dysfunction has been implicated as a contributing factor in diverse acute and chronic neurological disorders. However, its role in the epilepsy has only recently emerged. Metabolic changes during seizures have been observed in the brain. Seizures increase the metabolic rate for oxygen and glucose as well as cerebral blood flow. Ongoing seizures will lead to a sustained two to threefold increase in both the metabolic rate of oxygen and glucose. Furthermore the mitochondria include proteins, lipids, and DNA that could become targets of free radicals. These radicals may specifically inhibit the activity of the respiratory chain enzymes and also induce a transient change in permeability once the membrane has been depolarized. These interactions between free radicals and mitochondrial components reduce adenosine tri-phosphate (ATP) production and release excessive oxygenated radical capable of depleting energy and, subsequently, causing cell death (Fig. 2.5) (Cock, 2002; Arzimanoglou et al., 2003). The depolarization pattern of neurons during intense epileptiform activity causes elevation of intracellular calcium (Van den Pol, Obrieten, and Belousov, 1996) resulting in mitochondrial depolarization and intramitochondrial calcium accumulation (Duchen, 1992). This leads to energy failure and mitochondrial superoxide production (Schuchmann et al., 1999; Liang et al., 2000), which could trigger the acute neuronal cell death that occurs after status epilepticus (Fujikawa, Shinmei, and Cai, 2000). Deficiency of complex I of respiratory chain is documented in patients with temporal-lobe epilepsy (Kunz, Kudin, and Vielhaber, 2000). Furthermore Cock and coworkers (2002) have demonstrated a significant decrease in the activities of aconitase and α-ketoglutarate dehydrogenase, two mitochondrial enzymes known to be clinically sensitive to oxidative stress particularly involving nitric oxide, as well as reduced levels of glutathione, in the brain homogenates of status animal.

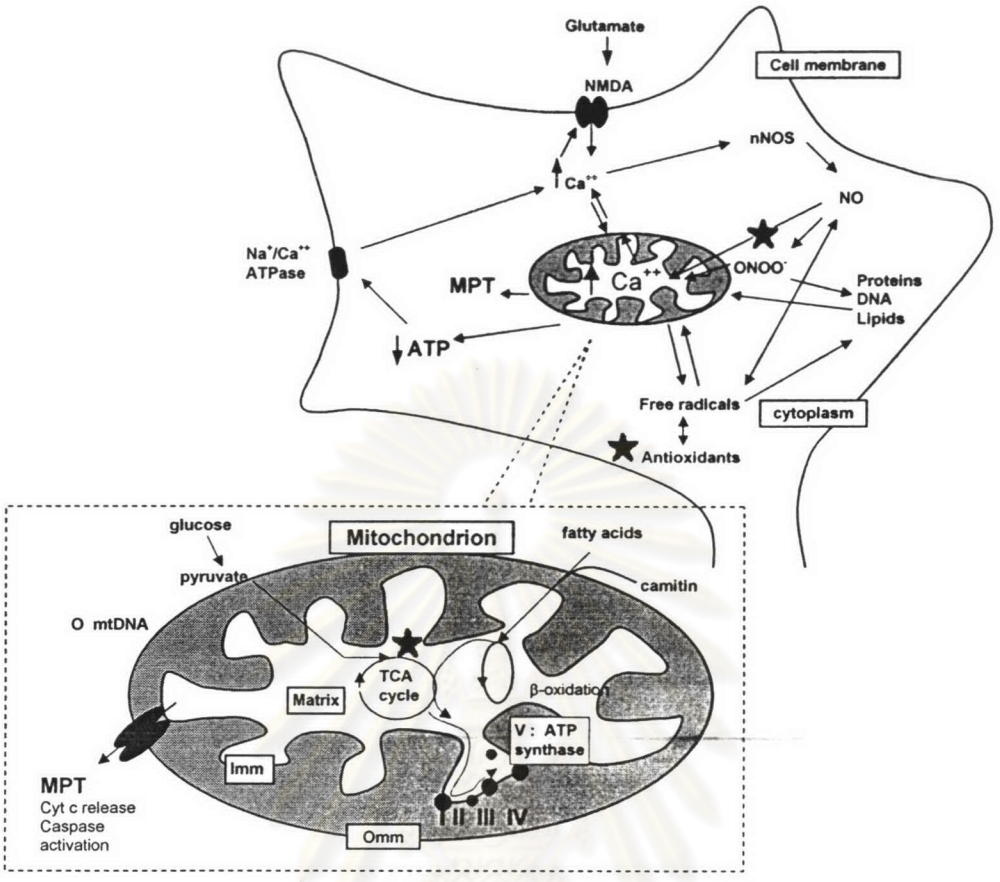


Figure 2.5 Schematic representation of major mitochondrial pathways involved in excitotoxic cell death. Glutamate activation at NMDA receptors results in calcium influx into the cell, and the mitochondrion. This activates nNOS, and increases nitric oxide (NO) production. High calcium and NMDA activation increase cellular energy requirements, involving activation of mitochondrial matrix enzymes involved in fuel breakdown (tricarboxylic acid (TCA) cycle and β-oxidation), including aconitase and α KDH. These feed electrons into the MRC (complexes I–V as marked in insert), which ultimately generates ATP. The mitochondrial respiratory chain (MRC) also produces free radicals, which may react with NO forming peroxynitrite ($ONNO^-$). Antioxidant systems, including glutathione, can inactivate low levels of free radicals, but in pathological conditions these may be overwhelmed and damage to cellular structures, including the MRC ensue. A damaged MRC produces more free radicals, and may also lead to mitochondrial permeability transition (MPT), and decreased ATP production. MPT activates apoptotic cell death, and ATP failure may further exacerbate problems with cellular calcium handling. Imm, inner mitochondrial membrane; omm, outer mitochondrial membrane; the data reported in this paper suggests dysfunction at the points marked with a ★ (Cock et al., 2002).

2.5 Treatment

Current epileptic treatment is focused on suppressing seizures and improving secondary effects of seizures. The goal of therapy is to keep the patient free of seizures without interfering with normal brain function. AED is one of the key management of epilepsy. Furthermore surgical treatment of epilepsy with resection of epileptogenic tissue or neurostimulation, e.g. vagus nerve stimulation, may be an alternative if AEDs fail (Foldvary, Bingaman, and Wyllie, 2001). Although epilepsy surgery is often considered the only causal treatment (or cure) of epilepsy, in most patients AED treatment has to be continued after surgery to achieve seizure control (Kwan et al., 2001).

2.5.1 Antiepileptic drugs

AEDs therapy is the mainstay of treatment of patients with epilepsy. The use of AEDs in the therapy of human seizure disorders is governed by their efficacy against specific seizure type. It is important to select an appropriate AED for the individual patients. The choice of antiepileptic drug should be based on the seizure classification, the age and sex of the patients, concurrent medical conditions, potential adverse effects, and the pharmacokinetic features of each drug (Dhillon and Sander, 1999; McNamara, 2001).

Over the last decade, there has been considerable progress in the pharmacotherapy of epilepsy, including the introduction of several new AEDs and improved formulations of older drugs (Bazil and Pedley, 1998; McCabe, 2000). The first generation of such drugs, some of which were discovered in the beginning of the twentieth century, include phenytoin, phenobarbital, benzodiazepines, and ethosuximide. The second generation of anticonvulsants include carbamazepine and valproate. These two generations of anticonvulsants have been studied and applied extensively by scientist and physicians. In addition, the third generation of anticonvulsants include vigabatrin, lamotrigine, gabapentin, tiagabine, topiramate, felbamate, levetiracetam, oxcarbazepine, and zonisamide (McAuley, Biederman, and Moore, 2002; Soderpalm, 2002). The main indications for the AEDs currently available were shown in Table 2.3 (McNamara, 2001).

In at least 60% of patients with epilepsy, the prognosis for seizure control is good. However, up to 40% of individuals with epilepsy suffer from intractable, e.g. pharmacoresistant epilepsy despite early treatment and an optimum daily dosage of an adequate AED (Regesta and Tanganelli, 1999; Kwan and Brodie, 2000). In addition to the problem of pharmacoresistance, several AEDs suffer from substantial problems with toxicity, particularly neurotoxic side effects and idiosyncratic reactions such as skin rash (Brodie, 2001). Thus, new AEDs with better safety, less toxicity, and higher efficacy in difficult-to-control patients are urgently needed.

Although the mechanisms of action of the currently marketed AEDs are still not completely understood, they ultimately involve alteration of the balance between neuronal excitation and inhibition (White, 1999). At the cellular level, three basic mechanisms are recognized (Table 2.4) (Kwan, Sills, and Brodie, 2001; Soderpalm, 2002).

The first is modulation of voltage-dependent ion channels (Na^+, Ca^{++}, K^+). Carbamazepine, lamotrigine, phenytoin, and valproic acid inhibit high-frequency firing at concentrations known to be effective at limitting seizures in human beings. Inhibiting of high-frequency firing is thought to be mediated by reducing the ability of Na^+ channels to recover from inactivation (McNamara, 2001; Soderpalm, 2002). Ethosuximide and valproate are known to treat absence seizure by inhibiting flow of Ca^{++} through T-type Ca^{++} channels. Phenobarbital inhibits voltage-activated Ca^{++} channels which one consequence of inhibition could be blockade of Ca^{++} entry into presynaptic nerve terminals and inhibition of release of neurotransmitters such as glutamate, resulting in net reduction of excitatory synaptic transmission (McNamara, 2001).

The second is direct or indirect enhancement of GABAergic neurotransmission. Benzodiazepines and barbiturates directly interfere in a positive manner with $GABA_A$ receptor function. The benzodiazepines increase the channel opening frequency, require to present of GABA to exert their effects. The barbiturates instead prolong the opening time of the channels, do not require GABA for producing their effects. Vigabatrin, valproate, and gabapentin are believed to indirectly enhance GABAergic

neurotransmission via enzyme inhibition, causing a reduced GABA metabolism and/or promoting GABA release through unknown mechanisms (Soderpalm, 2002).

The third category is inhibition of excitatory (particularly glutamate-mediated) neurotransmission (phenobarbital and valproate) via direct blockade of excitatatory receptors or alteration of glutamate metabolism or release (McNamara, 2001).

Table 2.3 Drugs for treatment of epileptic seizures (Modified from McNamara, 2001)

SEIZURES		ANTISEIZURE DRUGS	
		CONVENTIONAL	RECENT DEVELOPED
1. PARTIAL SEIZURES:	1.1 Simple partial seizure	Carbamazepine Phenytoin Valproate	Gabapentin Zonisamide Lamotrigine Topiramate Levetiracetam Tiagabine
	1.2 Complex partial seizure	Carbamazepine Phenytoin Valproate	Gabapentin Zonisamide Lamotrigine Topiramate Levetiracetam Tiagabine
	1.3 Partial with secondarily generalized tonic-clonic seizure	Carbamazepine Phenobarbital Phenytoin Valproate	Gabapentin Zonisamide Lamotrigine Topiramate Levetiracetam Tiagabine
2. GENERALIZED SEIZURES:	2.1 Absence seizure	Ethosuximide Valproate	Lamotrigine
	2.2 Myoclonic seizure	Valproate	Lamotrigine Topiramate
	2.3 Tonic–clonic seizure	Carbamazepine Phenobarbital Phenytoin Primidone Valproate	Lamotrigine Topiramate

Table 2.4 Proposed mechanisms of antiepileptic drug action (Kwan, Sills, and Brodie, 2001)

	Na^+ channels	Ca^{++} channels	K^+ channels	Inhibitory transmission	Excitatory transmission
Established AEDs					
Phenytoin	+++				
Carbamazepine	+++				
Esthosuximide		+++			
Phenobarbital		+		+++	+
Benzodiazepines				+++	
Sodium valproate	+	+		++	+
New AEDs					
Lamotrigine	+++	+			
Oxcarbazepine	+++	+	+		
Zonisamide	++	++			
Vigabatrin				+++	
Tiagabine				+++	
Gabapentin	+	+		++	+
Felbamate	++	++		++	++
Topiramate	++	++		++	++
Levetiracetam		+		+	

2.5.2 Surgical treatment

Epilepsy surgery is an effective alternative form of therapy for selected patients with intractable partial epilepsy (Radhakrishnan, So, and Silbert, 1998; Cascino, 2004), but resective surgery is restricted to patients with focal epilepsy in which the epileptogenic zone can be adequately identified (Foldvary, Bingaman, and Wyllie, 2001). Patients with medial temporal lobe epilepsy and lesional epilepsy may be favorable candidates for epilepsy surgery and have a surgically remediable epileptic syndrome (Cascino, 2004). The hallmark pathology of medial temporal lobe epilepsy is mesial temporal sclerosis. The surgically excised hippocampus in these patients almost invariably shows focal cell loss and gliosis (Cascino, Jack, and Parisi, 1992). Patients with lesional epilepsy may have a primary brain tumor, vascular anomaly or a malformation of cortical development (Cascino et al., 1992; Mosewich et al., 2000). The common surgical pathologies encountered in patients with lesional epilepsy include a low-grade glial neoplasm, cavernous hemangioma and focal cortical dysplasia (Awad et al., 1991).

The disabling effect of the seizures must be considered for each patient prior to considering surgical treatment. The frequency of seizure activity, type of seizures, co-morbidity, and underlying pathology are important variables that are used to determine the candidacy for surgical treatment. The psychosocial effects of the disease process should also be taken into account. The goals of epilepsy are to eradicate the seizure disorders, e.g. stop the seizures, avoid operative morbidity, and allow the individual to no longer be limited by the disease, e.g. work, attend school and/or live independently (Wiebe et al., 2001).

2.5.3 Vagus Nerve Stimulation (VNS)

In 1985, Zabara proposed that VNS might disrupt the hypersynchronous brain electrical activity that underlies epileptic seizures, and subsequent studies in animal models of epilepsy suggested that this was the case (Henry, 2002). Although the mechanism of action of VNS is still not fully understood, it was felt that continual stimulation of the vagus nerve by an implantable electrical device might result in widespread bilateral activation or deactivation of the brain circuits thought to be involved with epileptic seizures.

The reason for this lies in the neuroanatomical connections: afferent fibres from the vagus nerve comprise about 80% of the axons in the cervical vagus nerve, terminating on nuclei in the ipsilateral medulla and in the contralateral nucleus of the tractus solitarius (also in the brain stem). Inputs to these nuclei are then conveyed to widespread bilateral areas of the cerebral cortex, diencephalon and limbic system. The effects of VNS on these brain areas has been confirmed by positron emission tomography and functional magnetic resonance imagine studies (Henry, 2002). Vagus nerve stimulators are not on-demand devices like cardiac pacemakers, which are designed to abort an event. Rather, they mostly function interictally that is, between seizures, producing long-term alterations in the brain and thus decreasing seizure frequency. These long-term changes are not fully understood, as the characteristic interictal spike frequently seen on the electroencephalogram is largely unchanged.

2.6 Valproic acid (N-dipropylacetic acid) (VPA)

Thirty-five years since its introduction into clinical use, valproate (valproic acid) has become the most widely prescribed AED worldwide (Perucca, 2002). VPA is a simple branched-chain carboxylic acid (Fig. 2.6). The effectiveness of VPA as an anticonvulsant was discovered serendipitiously when other compounds were dissolved in VPA for administration to animals used in experimental models of epilepsy. Since then, VPA has been used to control a variety of seizures, including generalized, partial and absence seizures (Johannessen, 2000; and McNamara, 2001). In addition, a number of reports have also suggested that intravenous valproate could be of value in the treatment of convulsive and nonconvulsive status epilepticus. The most commonly reported adverse effects of valproate include gastrointestinal disturbances, body weight gain, tremor, hepatotoxicity and teratogenicity (McNamara, 2001; and Perucca, 2002). In addition, VPA can also suppress bone marrow function, especially thrombopoiesis, an effect that is generally reversed by discontinuing the drug, and can rarely cause aplastic anemia (Archarya and Bussel, 2000).

Although VPA is a broad spectrum anticonvulsant, the precise mechanisms by which it exerts its antiepileptic effects remain to be conclusively determined. Its

pharmacological effects involve a variety of mechanisms, including increased GABAergic transmission by involving metabolism of GABA, reduced release and/or effects of excitatory amino acids and blockade of voltage-gated sodium channels (McNamara, 2001; and Perucca, 2002). Another potential mechanism that may contribute to valproate's antiseizure actions is blocking of T-type calcium channels (McNamara, 2001).

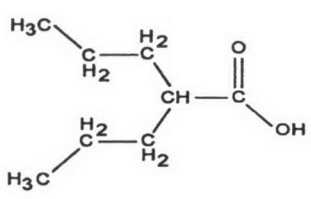
2.7 N(2-propylpentanoyl)urea (VPU)

VPU (Fig. 2.6) is a valproic acid analog which has been identified to possess an anticonvulsant activity. It demonstrated a higher protection than VPA in both the MES and the PTZ test, exhibiting an ED_{50} of 66 and 57 mg/kg, respectively. Furthermore, VPU was found to produce less neurological side effects and higher protective index (9.5) than did VPA (1.1) (Tantisira et al., 1997).

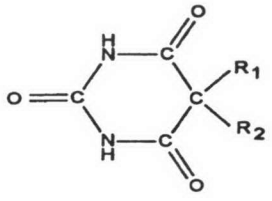
Brain microdialysis studies in anesthetized rats revealed that VPU significantly decreased the levels of cortical excitatory (aspartate and glutamate) and inhibitory (GABA and glycine) amino acid neurotransmitters in dose dependent manner . However, the depression was greatest on glutamate and least on glycine (Sooksawate, 1995).

By using microiontophoretic technique in anesthetized rats, it was found that VPU depressed spontaneous firing of both neurons of cerebral cortex and cerebellum (Khongsombat, 1997). Additionally, VPU at doses of 1-300 μM did not directly induce inward currents in acutely dissociated rat hippocampal pyramidal neuron. When coapplication of VPU with pentobarbital sodium, the increment potentiation of the $GABA_A$ currents by each of these drugs applied with GABA was elicited. These results show that the effect of VPU on the $GABA_A$ receptor may have some interaction directly or indirectly with the barbiturate site(s) on the $GABA_A$ receptor channel. This study suggested that the potentiation of the $GABA_A$ currents by VPU may, at least in part, contributes to its mechanism of anticonvulsant action (Jenthet, 2002). Recently, Ponsup (2003) reported that inhibitory effect of VPU on NR1A/NR2B NMDA receptors subtype by using the two-electrode voltage-clamp technique in *Xenopus laevis* oocytes, though rather weak, may be not the main anticonvulsant effect *in vivo*.

Developmental toxicity, regarding effects on axial rotation and embryonic growth, was lower in VPU-treated animals compared with those of VPA-treated. Furthermore hepatotoxic effects were observed *in vivo* and *in vitro* only in the large dose of VPU administration (Patchamart, 1996).



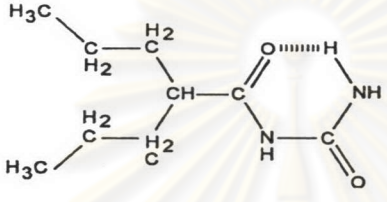
Valproic acid



Barbiturates

Pentobarbital: R1 = ethyl, R2 = 1-methylbutyl

Phenobarbital: R1 = ethyl, R2 = phenyl



N-(2-Propylpentanoyl) urea

Figure 2.6 The structures of valproic acid, barbiturates, and proposed structure of N-(2-propylpentanoyl) urea (modified from Saisorn, Patarapanich, and Janwitayanuchit, 1992)

2.8 Pilocarpine-induced seizure model

2.8.1 Behavioral changes in pilocarpine-induced seizure model

In 1983, Turski et al. demonstrated that systemic administration of pilocarpine, a cholinergic muscarinic agonist, produced electroencephalographic and behavioral limbic seizures in rats. These seizures were accompanied by widespread brain damage. Immediately after a pilocarpine injection, the animal was hypoactive. Subsequently, there was the appearance of facial automatisms, including chewing and eye blinking, followed by head nodding and motor limbic seizures (forelimb clonus, salivation and rearing on hindlimbs) which progressed to limbic SE (60-80 min). Long-term effects of pilocarpine administration to rats were characterized by three distinct phases; an acute period, a seizure-free (silent) period, and a period of spontaneous recurrent seizure. The acute period of 24-48 hrs duration corresponded to the pattern of repetitive limbic seizure and SE. A seizure-free (silent) period was characterized by a progressive return to normal EEG and normal behavioral of 4-44 days duration (mean 14 days). A period of SRS started 5-45 days after pilocarpine and persisted for at least 6 months or whole life of the animals (Turski et al., 1983; Leite et al., 2002; and Loscher, 2002).

2.8.2 Pilocarpine-induced neuronal damage

Brain damage after pilocarpine-induced status epilepticus, including those observed on hippocampal formation, thalamus, amygdala and neocortex is usually severe and widespread. The brain damage induced by status epilepticus in such preparations may be considered an equivalent of the initial precipitating injury event, usually a prolonged febrile convulsant, which is commonly found in patient with mesial temporal lobe epilepsy. The hippocampus is one of the most sensitive regions to epilepsy-related to brain damage following convulsions produced by pilocarpine and plays a major role in the development and maintenance of limbic seizures (Turski et al., 1983). At the electron microscopic level, the pilocarpine induced seizures caused neuronal damage that is similar to excitotoxic tissue damage caused by glutamate (Clifford et al., 1987). Covolan and coworkers (2000) demonstrated that a variety of morphologies ranging from apoptosis to necrosis, could be seen at 2.5 h after SE onset and continued at least over following 48 h. The extent of

neuronal damage in the hippocampal formation correlates directly with the duration of the initial SE (Lemos and Cavalheiro, 1995). Neuronal damage occurs within 20-40 min of status epilepticus (Fujikawa, 1996) and after 2 h it becomes irreversible, leading to a chronic epileptic condition (Lemos and Cavalheiro, 1995). In addition, pilocarpine-treated rats have reorganization of mossy fibers into the molecular layer of the fascia dentate that is similar to hippocampus from patients with hippocampal sclerosis (Leite, Garcia-Cairasco, and Cavalheiro, 2002). The mechanism of pilocarpine-induced SE and the produced neuronal damage is unclear.

2.8.3 Alterations of glutamate and GABA concentrations in pilocarpine-induced seizure model

Many microdialysis studies demonstrated significant alterations of glutamate and GABA concentrations in pilocarpine-induced seizure (Walton et al., 1990; Cavalheiro et al., 1994). Smolders et al. (1997) reported that after ceasing the intrahippocampal administration of pilocarpine, the decrease in glutamate, aspartate and GABA release was followed by a significant elevation of the extracellular levels of both excitatory and inhibitory amino acids in the hippocampus of freely moving rats. They suggested that changes in extracellular hippocampal amino acid levels (glutamate, aspartate and GABA) were not involved in seizure onset, but might play a role in seizure maintenance and spread of seizures which continued until the end of the experiment. These reports supported the hypothesis of the cholinergic system being responsible for seizure onset and maintenance, and of driving amino-acid mechanism to support sustained seizure activity with neuronal damage (Turski et al., 1989; Smolders et al., 1997).

2.8.4 Oxidative stress and mitochondrial dysfunction in pilocarpine-induced seizure model

In pilocarpine model, there is a great involvement of excitotoxic neuronal injury (Cavalheiro et al., 1991; Mello et al., 1993; and Bonan et al., 2000). ROS production has been considered to be a part of mechanisms involved with the glutamatergic excitotoxicity *'in vitro'* (Bonfoco et al., 1995) and *'in vivo'* (Bruce and Baudry, 1995; Ueda et al., 1997; and Shulz et al., 1995). In hippocampus there was a slight enhancement in the

level thiobarbituric acid reactive substances levels measured 12-14 h after the end of status epilepticus induced by pilocarpine (Dal-Pizzol et al., 2000). This result demonstrated that, pilocarpine induced status epileticus is followed by changes in the level of lipid peroxidation in the hippocampus. These suggested that ROS could be involved in the neuronal damage induced by pilocarpine. In addition, the superoxide dismutase activity was decreased in association with high hydroperoxide concentration after long-lasting status epilepticus period induced by pilocarpine (Bellissimo et al., 2001). Kudin and coworkers (2002) demonstrated seizure-dependent changes in mitochondrial oxidative phosphorylation in the epileptic rat hippocampus. Pilocarpine-treated rats exhibited a selective decline of the activities of complex I and complex IV of the respiratory chain in the CA3 and CA1 hippocampal pyramidal subfields.

The distinct features of an acute damage induced by status epilepticus, a silent interval between injury and the onset of spontaneous seizures, and a chronic epilepticus state have allowed the possibility to study AEDs for different purposes, a) in the acute phase, identification of compounds with the efficacy against refractory status epilepticus and neuroprotection against damage induced by sustained seizure; b) in the latent period, identification of agents with a potential for preventing epileptogenesis and against seizure-induced long-term behavioral deficits; c) in the chronic phase, testing drugs effective against spontaneous partial and secondarily generalized seizures (Leite, Garcia-Cairasco, and Cavalheiro, 2002)

The present study was aimed to determine

- anticonvulsant activity of VPU and VPA against pilocarpine-induced seizure
- effects of VPU and VPA on neuronal cell loss in hippocampus (CA1 and CA3 regions)
- effects of VPU and VPA on neurochemical changes (excitatory and inhibitory amino acid neurotransmitter in the hippocampus)
- effects of VPU and VPA on lipid peroxidation
- effects of VPU and VPA on neuronal mitochondrial function