

Novel Neuroprotective Strategies and Targets of Intervention in Epilepsy

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1. Introduction

Epilepsy is a debilitating disorder that affects over 50 million people worldwide, resulting in \$15.5 billion in medical expenses and lost income/worker productivity in the United States every year (Patel, 2004). Epilepsy, also known as *status epilepticus* (SE), is described as the unregulated over stimulation of neurons throughout various regions of the brain. SE is characterized by seizures lasting for 30 or more minutes accompanied by a loss of consciousness. This disorder has been associated with significant rates of morbidity and mortality, possibly induced by neuronal damage and dysfunction (Sleven, et al., 2006). It is thought to be the result of an imbalance of excitatory and inhibitory input in a subset of neurons, which is then propagated to other regions of the brain, causing improper activation of multiple brain regions and uncontrolled cortical output (Rho, et al., 2004). Most patients are either under the age of 20 or over 65 years old, with a greater prevalence being in younger patients. While development of SE has a wide range of possible etiologies, whether spontaneously, as the direct result of trauma, brain tumors, metabolic abnormalities, or due to genetic predisposition, the exact mechanism(s) of the development of SE is poorly understood (Pellock, et al., 2001, Rho, et al., 2004).

Oxidative stress has been associated with SE; however, it continues to be somewhat controversial whether it plays a causal role in the development of epilepsy or if it is simply the consequence of prolonged excitation (Patel, 2004). This increased excitation exerts high metabolic demands on cellular systems, such as Na^+/K^+ pumps and other ATP dependent mechanisms, required for maintaining normal cellular homeostasis. Mitochondria are the main source of ATP in neurons and mitochondrial dysfunction has been linked to many acute and chronic neurological disorders including Parkinson's disease, traumatic brain injury, stroke/ischemia, and Alzheimer's disease.

Mitochondrial dysfunction is known to increase oxidative damage via increased mitochondrial reactive oxygen species (ROS) production, which has been shown to be a critical side effect of prolonged epileptic seizure and may cause increased susceptibility to subsequent seizures (Patel, 2002). It has also been shown that after prolonged seizure activity there is significant oxidative damage to mitochondrial DNA (mtDNA), which is responsible for encoding key proteins of the electron transport chain (ETC) required for oxidative phosphorylation and normal mitochondrial function (Patel and Li, 2003). Disruption of ATP production can cause impaired mitochondrial and plasma membrane transporter function, initiation of necrotic

pathways, alteration of neurotransmitter metabolism, and opening of the mitochondrial permeability transition pore (mPTP), ultimately leading to cellular destruction/dysfunction (Patel and Li, 2003, Sullivan, et al., 2005).

2. GABA

Gamma-aminobutyric acid (GABA), the primary inhibitory neurotransmitter in the brain, activates Cl⁻ and HCO₃⁻ (GABA_A) or K⁺ (GABA_B) permeable receptor ligand-gated channels by binding to specific GABAergic receptors on cellular membranes of neurons, thereby hyperpolarizing the cell and rendering it unable to fire rapid sequential action potentials (Czapinski, et al., 2005, Kwan, et al., 2001, Rho, et al., 2004). Deficiencies in these receptors are believed to play an important role in the development of epilepsy, as studies using various models of epilepsy indicate that modulation associated with subunits of the GABA_A receptor cause a decreased ability to inhibit neuronal activity and cause promotion of neuronal hyperexcitability (Brooks-Kayal, et al., 1998, Rho, et al., 2004). There is also some evidence that the developmental roles of these receptor subtypes are not solely associated with the inhibition of neurons and, in the case of GABA_A receptors, they can also act as excitatory inputs via GABA activation in immature neuronal networks.

GABA_B receptors are believed to be responsible for primary inhibitory effects at this early stage of development, although they may not be able to compensate for increases in excitation (Pellock, et al., 2001). The loss of GABAergic neurons and the subsequent improper neuronal compensatory reorganization for the lost inhibitory input or the loss of key GABA regulatory enzymes could alter the excitatory/inhibitory balance and lead to inappropriate excitatory signal propagation initiating SE (Rho, et al., 2004). Improper potassium regulation has also been suggested to be a potential cause of the increased excitability in young neurons due to its decreased clearance from the extracellular environment evoking repetitive neuronal discharges (Pellock, et al., 2001). Studies using calcium chelators (i.e. BAPTA) suggest that the loss of inhibitory neurons is due to their inability to properly buffer calcium, rendering these neurons unable to maintain adequate membrane potential, which could implicate mitochondrial involvement (Rho, et al., 2004).

However, calcium has also been suggested to have age-specific effects on NMDA receptors by acting as the regulatory ion, rather than magnesium (Mg²⁺), due to its increased influence on the development of neuronal networks in the developing brain, and increased levels of intracellular calcium may interfere with the ability of immature neurons to make appropriate inhibitory connections during this critical period (Pellock, et al., 2001). This aberrant Ca²⁺ cycling effect highlights the importance of mitochondrial homeostasis due to their function of Ca²⁺ sequestration, which regulates the cytosolic concentrations in order to maintain proper cellular function.

3. Excitotoxicity

Recent studies have shown seizures to be associated with neuronal loss in various regions of the brain, including age-dependent damage to hippocampal regions; and it has been suggested that this damage is a result of prolonged excitation by excitatory amino acid (EAA)-induced excitotoxicity (Pellock, et al., 2001, Sullivan, 2005) (Fig. 1). During seizures neurons become depolarized for a prolonged period of time resulting in an increase in Na⁺ influx through voltage-dependent channels, and this prolonged increase in Na⁺ perpetuates neuronal depolarization. This increased and sustained depolarization causes the voltage

dependent Mg^{2+} block to be removed from NMDA channels, allowing them to be activated by glutamate thus facilitating the influx of Ca^{2+} and a loss of neuronal Ca^{2+} homeostasis (Pellock, et al., 2001, Rajasekaran, 2005, Sullivan, 2005).

Whether oxidative stress is the cause or consequence of prolonged activation by EAA has been controversial. Studies suggest that chronic seizures result in increased oxidative stress, upregulation of neurotrophic factor genes, and structural rearrangement, all of which can contribute to increased susceptibility by inducing a chronic state of hyper-excitability (Liang and Patel, 2004, Patel, 2002). Key glial transporters (GLT-1 and GLAST) responsible for the uptake of exogenous glutamate from the extracellular environment can also be damaged by oxidative stress, resulting in the propagation and extension of activation by this EAA (Liang and Patel, 2004).

4. Mitochondria

Mitochondria function primarily as the major source of the energy production for the cell and are responsible for maintaining calcium homeostasis by sequestering excess calcium from the cytosol. The mitochondria perform these vital functions by shuttling electrons down a series of complexes in the inner mitochondrial membrane called the electron transport chain (ETC) and subsequently pump protons across the inner membrane from the matrix creating a membrane potential ($\Delta\Psi$) within the inner membrane space (Figure 2).

This membrane potential can be used to sequester calcium through membrane channels and to carry out oxidative phosphorylation by complex V (ATP synthase) to produce ATP (Brookes, et al., 2004, Nicholls and Budd, 2000, Sullivan, et al., 2002, Sullivan, et al., 1998). A normal byproduct of oxidative phosphorylation is the production of ROS, which under normal physiological circumstances is scavenged by endogenous antioxidant systems such as MnSOD, Cu/ZnSOD, and glutathione (GSH) (Ilhan, et al., 2005). However, during trauma or prolonged epileptic seizure the production of ROS can overwhelm the endogenous antioxidant defense systems and cause damage to lipids, proteins, and DNA resulting in cellular dysfunction and subsequent neuronal loss.

The brain is both rich in mitochondria and substantially more sensitive to insult and oxidative stress than any other tissue in the body because of its high metabolic demand for oxygen and glucose, its large amount of peroxidizable membranous polyunsaturated fatty acids (PUFA), poor repair/regenerative mechanisms, and high iron content (Patel, 2002, Rho, et al., 2004). In addition to epilepsy there has been an association between both oxidative damage and mitochondrial dysfunction with the development of many cognitive disorders, including Parkinson's and Alzheimer's disease (Ilhan, et al., 2005, Patel, 2002, Sullivan, et al., 2004). Studies showing that oxidative damage precedes seizure initiation and studies implementing strategies to limit free radical formation and several antioxidant therapies have indicated that oxidative mechanisms are involved a causal role of seizure induced neuronal loss. However, studies have also detected oxidative damage to mitochondrial complex I, as well as some integral citric acid cycle proteins, up to 44 hours after SE, suggesting that oxidative damage is the result of prolonged seizure activity (Gibbs, et al., 2006, Jung, et al., 2001, Patel and Li, 2003, Patel, 2002, Rong, et al., 1999).

5. Antioxidative mechanisms

Superoxide dismutase (SOD), a major component of the endogenous antioxidant system of the cell, has three isoforms which each have distinct localizations within the cell.

Cu/ZnSOD (SOD1) is primarily found in the cytosol, MnSOD (SOD2) is found in the mitochondria, and EC-SOD (SOD3) is found in the extracellular space (Patel and Li, 2003). These enzymes catalyze the dismutation of superoxide into hydrogen peroxide (H_2O_2) and oxygen (O_2) at a rate very close to its diffusion rate (Ilhan, et al., 2005, Patel and Li, 2003). Glutathione peroxidase (GPx) and glutathione (GSH), another major component of the endogenous antioxidant system, catalyze H_2O_2 into water preventing the formation of hydroxyl radicals, rendering the previously dangerous superoxide species harmless to cellular structures (Ilhan, et al., 2005, Rho, et al., 2004).

Glutathione levels were shown, *in vitro* and *in vivo*, to decrease as early as 4 hours after SE, which highlights its importance in influencing mitochondrial /cellular damage outcome after SE (Gibbs, et al., 2006, Sleven, et al., 2006). Studies conducted by modulating the level of SOD in a mouse model of epilepsy have given us insights into the role of antioxidant systems in the prevention of oxidative stress and a seemingly causal role of oxidative damage in seizure. Homozygous MnSOD $-/-$ knockout mice are embryonic lethal, which highlights its vital function in physiological function and developmental processes. Using heterozygously expressing ($-/+$) or transgenic overexpressing MnSOD mice have allowed for the investigation of the consequences of diminished or overabundant (respectively) antioxidant capacity on seizure development and hippocampal damage. It has been shown that overexpression of MnSOD, 0.5-2 fold, can attenuate kainate induced seizures, however animals with diminished MnSOD levels showed an exacerbation of kainate-induced seizure and hippocampal damage, which was attenuated with antioxidant treatment (Patel, 2002). Overexpression of MnSOD also produces lower amounts of inactive aconitase and 8-hydroxy-2-deoxyguanosine (8-OHdG), measures of oxidative protein and DNA (most likely mtDNA) damage, indicating a role in the preservation of mitochondrial function (Gonzalez, et al., 2005, Patel, 2002, Sleven, et al., 2006).

Damage to mitochondrial complex I, α -keto-glutarate dehydrogenase, citrate synthase, aconitase, and GSH can be detected at time points well after the end of an epileptic episode, and damage to these cellular components can induce cell death cascades and increase the likelihood of future seizures (Gibbs, et al., 2006). Highlighting the importance of this oxidative damage in epileptogenic pathologies is the specific defect of complex I activity found in the hippocampal CA3 region of patients suffering from hippocampal sclerosis and intractable seizures, which was found to be sufficient enough to affect ATP production in this region, possibly accounting for the pathology of seizure development in these patients (Gibbs, et al., 2006, Kunz, et al., 2000).

6. Antiepileptic drugs

AEDs have been in use for the attenuation of seizures since the early part of the 20th century (Fig. 2). Since their inception, many pharmacological interventions have been examined for their efficacy in attenuating the development of epileptic seizures, however, out of the thousands of compounds that have been screened for their ability to treat seizure, only a handful of antiepileptic drugs have been approved for clinical use. It was believed that early AEDs, such as benzodiazepines, phenobarbital, and valproate decreased seizure prevalence by increasing GABA inhibition of aberrant neuronal excitation, where as newer AEDs affect a much broader set of cellular systems, which can increase the complexity of pharmacological effect (Czapinski, et al., 2005, Kwan, et al., 2001). In addition to the potential detrimental effect, most of these interventions focus on the prevention of future

seizures rather than preventing neuronal damage resulting from prolonged or multiple seizures. Alternative targets at the cellular level to modulate seizure incidence and, perhaps more importantly, attenuate neuronal damage must be developed in order to create a more substantial and permanent treatment for epilepsy.

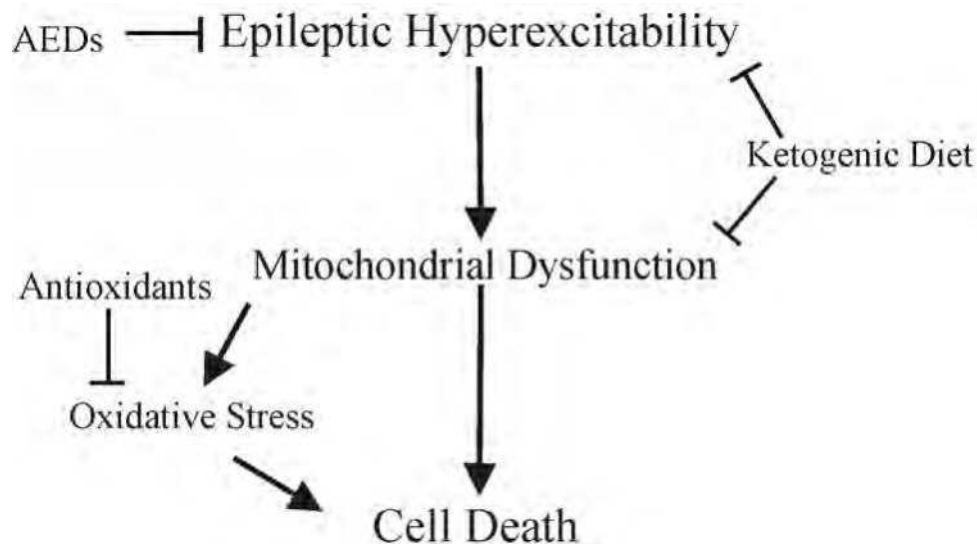


Fig. 1. Hypothetical sites of neuroprotective actions of epileptic therapeutic strategies. Following seizure mitochondria buffer rises in intracellular Ca^{2+} . Excessive mitochondrial Ca^{2+} cycling results in an increase in ROS production and in the initiation of cell death. AEDs target hyperexcitability by two mechanisms; I.) increasing inhibitory neurotransmission or II.) decreasing excitatory neurotransmission. The ketogenic diet alters neurotransmitter metabolism which decreases neuronal excitability. Additionally, the ketogenic diet has been shown to increase uncoupling protein activity and in-turn reduces oxidative stress. Antioxidants act to decrease oxidative stress and prevent cell death.

6.1 GABA modulators

AEDs that modulate the action of GABA in order to increase the inhibitory effect of this neurotransmitter have been widely used as the first line of treatment therapies for SE (Gibbs, et al., 2006). These drugs include Phenobarbital (PB), Benzodiazepines (BZD), Vigabatrin (VGB), and Tiagabine (TGB). PB, perhaps the oldest and most studied member of the barbiturate family has been used since the turn of the 20th century for its properties as an anticonvulsant and sedative, confers anticonvulsant protection to animals subjected to various experimental seizure models (Kwan, et al., 2001). This type of GABA modulators include that also includes Methylphenobarbital, Pentobarbital, and Primidone. In addition to increasing the affinity of GABA for its respective receptor and the activation of chloride channels, PB extends the time of chloride channel opening, without affecting frequency of opening or channel conductance (Czapinski, et al., 2005, Kwan, et al., 2001). PB has also been shown to elicit their antiepileptic effect by directly blocking high-voltage-activated Ca^{2+}

channels and inhibiting AMPA/kainate receptors, preventing depolarization of neurons, propagation of the aberrant signals, and the cascade of damaging secondary events within the cell (Czapinski, et al., 2005, Kwan, et al., 2001, Pellock, et al., 2001, Sullivan, 2005). This AED is unique due to its ability to activate GABA_A receptors in the absence of exogenous GABA, and that this augmentation of GABA-mediated inhibition and inhibition of glutamate-mediated excitation is selective for the postsynaptic terminal (Kwan, et al., 2001, Pellock, et al., 2001). It has also been suggested that PB locks Na⁺ channels, together with its modulation of GABA receptors it induces its anticonvulsive action by inhibition of glutamate activation (Pellock, et al., 2001, Rho, et al., 2004, Sullivan, 2005, Trojnar, et al., 2002). PB has showed great efficacy in attenuating seizure and is generally a safe medication with a prolonged treatment duration of action, however, there are still cognitive and behavioral side effects, as well as increased hepatic enzyme activation effecting the concomitant administration of additional AEDs, limiting its use in some situations (Gibbs, et al., 2006, Pellock, et al., 2001). There have been conflicting studies describing PB as both neuroprotective and neurodegenerative after SE, however its neurodegenerative effect may be isolated to the developing brain where mitochondrial degeneration, deficits in hippocampal based behavior measurements, and myelin degradation have been found with PB administration early in life (Sankar and Holmes, 2004, Trojnar, et al., 2002).

Along with PB, Benzodiazepines, which have more than 50 distinct family members including Diazepam, Lorazepam, Midazolam, and Clonazepam, represent the first line treatments for SE and have a broad spectrum of clinical activity used mainly for partial and idiopathic generalized epilepsies, complex seizures, secondary generalized motor seizures, and acute SE (Gibbs, et al., 2006, Pellock, et al., 2001). This class of drugs also bind to the GABA_A receptor subtype at the allosteric binding site on the α -subunit inducing an increase in the frequency of Cl⁻ channel opening, however they are unable to activate these receptors in the absence of endogenous GABA (Czapinski, et al., 2005, Gibbs, et al., 2006, Granja, et al., 1997, Pellock, et al., 2001). It has also been shown that BZDs can also block Na⁺ channels at high concentrations encountered during intensive treatment of acute SE (Czapinski, et al., 2005). They work to lower seizure threshold in order to decrease the duration of erroneous discharges thereby limiting the spread of the aberrant excitation to adjacent brain regions. This type of AED is marked for its consistency in efficacy, however they are susceptible to tolerance development and have been shown to exacerbate neuronal damage in some experimental models, limiting their use in chronic seizure disorder patients (Gibbs, et al., 2006, Pellock, et al., 2001). Much like the action of PB, these drugs seem to have an altered neuroprotective function depending on neuronal development, where as in mature animals BZDs have been shown to be neuroprotective, in immature animals these same compounds show a dose-dependent induction of apoptotic cell death (Sankar and Holmes, 2004, Sullivan, 2005, Trojnar, et al., 2002).

Vigabatrin and Tiagabine were designed as a new generation of novel AED intended to regulate GABA metabolism by either acting as an irreversible inhibitor of (GABA-T) GABA transaminase (VGB) or inhibiting glial/neuronal uptake of GABA (TGB) both resulting in increased and prolonged duration of GABA signaling (Czapinski, et al., 2005, Kwan, et al., 2001, Pellock, et al., 2001, Trojnar, et al., 2002). VGB, a structural GABA analogue, actually uses GABA-T to enzymatically transform in to its active metabolite, which then irreversibly binds to GABA-T and acts to inhibit its ability to degrade GABA, causing a prolonged increase in GABA levels throughout the brain, without manipulating any other GABA

synthesis or metabolic enzymes (Kwan, et al., 2001). TGB inhibits GABA reuptake from the synaptic cleft by selectively blocking the GAT-1, a GABA transporter, without affecting GAT-2, GAT-3, or GBT-1; allowing its effects to be localized primarily to the cerebral cortex and the hippocampus (Kwan, et al., 2001). Both TGB and VGB were designer drugs targeted at specific aspects of the GABAergic system and have been shown to be protective against seizure and neurodegeneration; however, like PB and BDZ there is also evidence that they are detrimental to the developing nervous system (Kwan, et al., 2001, Pellock, et al., 2001, Trojnar, et al., 2002). VGB administration for refractory epilepsy in children and infantile spasms has presented a pronounced prevalence (~40%) retinal toxicity and development of visual field defects, and as a result its use has declined worldwide in younger patients (Czapinski, et al., 2005, Kwan, et al., 2001, Pellock, et al., 2001).

With any AEDs whose primary action is prolonging the duration of inhibition by the GABAergic system, symptoms such as, drowsiness, dizziness, agitation, amnesia, fatigue, depression, weight gain, ataxia, and nystagmus are prevalent in patients with prolonged use. These drugs have been shown to be efficacious in attenuating seizures and have shown potential in promoting neuroprotection; however, the key to their effectiveness will be the regulation of their administration to children due to their age specific effects on the developing brain.

6.2 Ion channel modulators

Recently developed AEDs have been designed to modulate specific ion channels to prevent aberrant and prolonged excitation. Na⁺ channels blockers such as Phenytoin (PHT) and Carbamazepine (CBZ), Ca²⁺ blockers such as Ethosuximide (ESM), or Na⁺/Ca²⁺ (L-Type) channel blockers such as Lamotrigine (LTG), Oxcarbazepine (OXC), and Zonisamide (ZNS), were introduced to replace the sedative GABAergic modulating AEDs (Czapinski, et al., 2005, Kwan, et al., 2001, Pellock, et al., 2001, Sullivan, 2005). Blockage of Na⁺ channels reduces the ability of neurons to undergo multiple rapid excitations resulting in increased instances of prolonged depolarization propagated by the activation of voltage-dependent Na⁺ channels, and increased cellular swelling via Cl⁻ influx, ultimately leading to cellular damage and dysfunction (Kwan, et al., 2001, Sullivan, 2005). Increased Ca²⁺ influx is also a result of prolonged excitation, which causes increased excitatory amino acid (EAA) release from the presynaptic membrane into the synaptic cleft, resulting in further dissemination the aberrant excitatory activation to surrounding brain regions, inducing cellular damage via secondary signaling cascades (Brookes, et al., 2004, Kwan, et al., 2001, Nicholls and Budd, 2000, Nicholls and Ferguson, 2002, Pellock, et al., 2001).

PHT and CBZ share the selective mechanism of Na⁺ channel inhibition, which decreases the frequency of depolarization, thereby decreasing the amount of irregular signal transmissions. In the case of PHT, it is the most effective when there is a high frequency of depolarization, which is an important feature of this mechanism; instead of completely inhibiting activation it works to minimize only excessive neuronal activity (Kwan, et al., 2001). Also, PHT is generally the most well tolerated AED, side effects are common due to its unique non-linear elimination kinetics, but these symptoms can normally be attenuated with proper dose adjustments (Pellock, et al., 2001). There are also a few reports suggesting that PHT functions by blocking high voltage Ca²⁺ channels and may be involved in GABAergic modulation, however this evidence has yet to be fully substantiated (Granger, et al., 1995, Kwan, et al., 2001, Rowley, et al., 1995). CBZ, which is effective in focal (partial)

and grand mal (tonic-clonic) seizures and exclusively blocks Na^+ channels, seems to be less effective as a neuroprotective agent than other anticonvulsant compounds in treating SE, but was shown to have increased protection for ischemia/traumatic insult (Czapinski, et al., 2005, Pellock, et al., 2001). Neurotoxicity of CBZ, like PHT, was only found when the administered dose was at supra-therapeutic concentrations (Czapinski, et al., 2005, Pellock, et al., 2001, Sullivan, 2005).

OXC is a structural analogue of CBZ, however due to modifications designed to prevent the production of the 10,11-epoxide metabolite, it is more easily tolerated by the patient and shows a decreased level of side effects compared to CBZ (Kwan, et al., 2001, Pellock, et al., 2001). It has the ability to block both Na^+ and L-type Ca^{2+} channels, and has an additional possibly unique function of increasing K^+ channel conductance, all of which leads to decreased excitation and excitotoxic signaling cascades (Czapinski, et al., 2005, Kwan, et al., 2001, Pellock, et al., 2001). Retigabine (RTB) functions as an anticonvulsant by decreasing the activation threshold of neurons via activation of K^+ channels and increasing GABA-mediated Cl^- currents (Czapinski, et al., 2005). ZNS, which is one of the only drugs specifically evaluated for the pediatric patient population, also blocks both Na^+ and Ca^{2+} channels, GABA receptor linked Cl^- channels, enhances dopaminergic/serotonergic neurotransmission, and inhibits glutamate-induced excitation (Czapinski, et al., 2005, Kwan, et al., 2001, Pellock, et al., 2001). In addition to its antiepileptic and anticonvulsant effects, ZNS also decreases the production of exogenous nitric oxide and free radicals, giving it a unique neuroprotective quality against oxidative stress resulting from prolonged SE (Czapinski, et al., 2005). LTG is a novel AED, effective in blocking both Na^+ (primarily slow inactivated state) and L-type Ca^{2+} channels, which is efficacious in treating partial, absence, myoclonic and tonic-clonic seizures (Kwan, et al., 2001, Pellock, et al., 2001, Trojnar, et al., 2002). ESM is unique from other ion channel modulators in that it is specific for T-type Ca^{2+} channel blockage, and does not have any other known mechanism (Kwan, et al., 2001). It has been used for many years for generalized absence seizures, due to its ability to prevent the characteristic T-type Ca^{2+} channel induced synchronized 3-Hz spike-and-wave discharge (Kwan, et al., 2001).

6.3 Multi-mechanistic AEDs

There have been many AEDs developed with multiple mechanisms of action to attenuate seizure activity. One of the most studied and widely used multi-mechanistic AED is valproic acid (VPA), however, the exact mechanism of its anticonvulsant action is still debated, and in fact may be a number of different mechanisms (Kwan, et al., 2001, Pellock, et al., 2001). It has proven to be effective in treating a range of disorders in addition to epilepsy, including bipolar affective disorder and migraine headaches (Schulpis, et al., 2006). The possible mechanisms include, the modulation of the GABAergic system by modulation of either inhibition of GABA-T and succinic semialdehyde dehydrogenase or the increase of glutamic acid dehydrogenase, which work to either inhibit GABA breakdown or elevate GABA synthesis (respectively), however the later is thought unlikely to be the primary mechanism (Czapinski, et al., 2005, Pellock, et al., 2001). VPA has also been shown to block voltage-dependent Na^+ channels, thereby reducing sustained repetitive firing of neurons, however it does not exert an effect on the recovery of Na^+ channels from the inactivated state (Kwan, et al., 2001, Pellock, et al., 2001). This AED also has similar effects on T-type Ca^{2+} channels as does ESM, which may account for its efficacy in specifically treating

absence seizures (Kwan, et al., 2001, Trojnar, et al., 2002). However, recent studies have shown that chronic VP administration can cause some side effects including increased free radical formation (ROS) and oxidative DNA damage, impairment of liver mitochondrial function, hepatotoxicity, and increased serum lipids, lipoproteins and Apo lipoproteins increasing the risk of cardiovascular problems (Karikas, et al., 2006, Schulpis, et al., 2001, Schulpis, et al., 2006).

GBP is a structural analogue of GABA designed to be a blood brain barrier permeable mimetic of GABA activation of GABA receptors inducing an increase in the activation of the GABAergic system. However, studies using binding assays show that there is not affinity for GBP for either GABA_A or GABA_B receptors; instead there is evidence that GBP acts through interactions with the L-amino acid transport system, reducing high frequency action potential firing via Na⁺ channel blockage, possible modulation of GABA metabolism, and blockage of L-type voltage dependent Ca²⁺ channels (Czapinski, et al., 2005, Kwan, et al., 2001, Pellock, et al., 2001). Both Felbamate (FBM) and Topiramate (TPM), also multi-mechanistic AEDs, can inhibit neuronal activation by the EAA glutamate (Czapinski, et al., 2005). FBM, which is used clinically to treat a wide variety of seizure disorders, can have dual actions on excitatory and inhibitory neuronal mechanisms, as highlighted by the conflicting studies showing the lack of ligand binding to the GABA receptor and increases in GABA-mediated responses, as well as the inhibition of NMDA-linked excitation (Pellock, et al., 2001). TPM, however, has clearly been shown to block Na⁺ channels, increase GABAergic-mediated inhibition, and antagonize glutamate activation of both NMDA and AMPA/kainate receptors (Pellock, et al., 2001). Both FBM and TPM have good clinical efficacy in reducing seizure and have a low incidence of tolerance development, which makes these AEDs more ideal for the treatment of chronic epileptic disorders.

6.4 Unknown mechanism

There are still more compounds being investigated for their potential antiepileptic properties, however the mechanism of some of these AEDs have not fully been elucidated. One such compound, Levetiracetam (LEV), is an analogue of piracetam and has been used and has shown great promise as an anticonvulsant pharmacological therapy; however, its exact mechanism of conferring this anticonvulsant action is currently known. Studies have shown that LEV has little to no effect on increasing the inhibitory effect of the GABAergic system, and it has been inferred that it has a completely unique activity profile that is unlike any of the AEDs mechanisms that have come before it, including Na⁺ and Ca²⁺ channel blockage, K⁺ channel activation, and GABA/glutamate system modulation (Gibbs, et al., 2006, Kwan, et al., 2001, Pellock, et al., 2001). Perhaps the most interesting property of LEV is that it has exhibited neuroprotective effects after experimentally induced SE; and has been shown to attenuate seizure severity. In addition to this attenuation, decrease damage to many vital mitochondrial proteins, such as α -keto-glutarate dehydrogenase, complex I, Aconitase, citrate synthase, and GSH has been shown with LEV treatment; which seems indicate an ability to attenuate oxidative damage and mitochondria dysfunction resulting from prolonged seizure induced excitation (Kwan, et al., 2001, Mazarati, et al., 1998). This suggests that LEV acts to not only prevent the damaging epileptic episode, but also to promote intracellular/intramitochondrial reparative mechanisms. The elucidation of this mechanism of anticonvulsive neuroprotection is could result in a wider administration of LEV, and has the potential to lead to the development of an entirely new class of AEDs with similar neuroprotective effects.

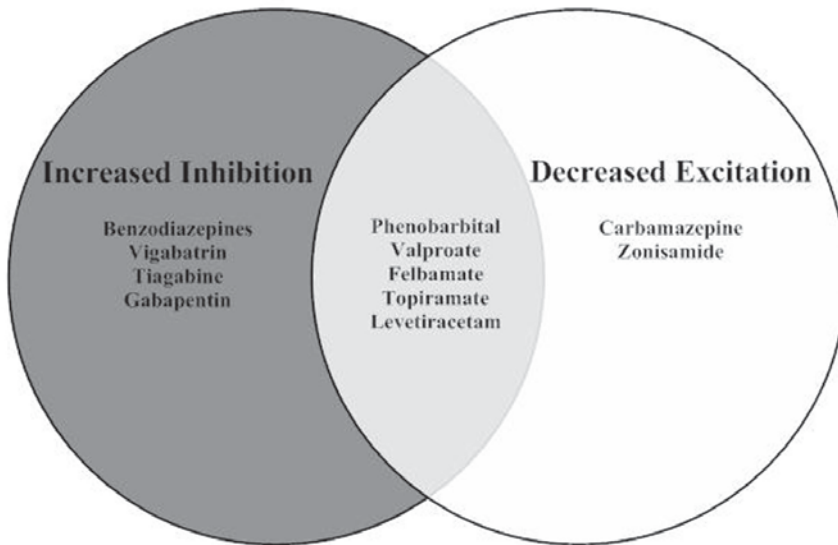


Fig. 2. Effects of commonly used AEDs.

7. Antioxidants

Antioxidant therapy following seizure has been shown to be beneficial (Acharya, et al., 2008). Resveratrol is a naturally occurring antioxidant and has been shown to decrease hippocampal neuronal cell death and decrease mossy fiber sprouting following kainite-induced temporal lobe epilepsy (Wu, et al., 2009). Other antioxidants, such as ascorbic acid and α -tocopherol, have been shown to decrease neuronal cell death following seizure induced with pilocarpine (Tome Ada, et al., 2010). Additionally, the naturally occurring antioxidant melatonin has been shown to be neuroprotective in human epilepsy (Molina-Carballo, et al., 1997). Thus, oxidative stress is one potential target for neuroprotective intervention following seizure.

8. Alternative non-pharmacological treatments

An alternative to pharmacological interventions is the implementation of the ketogenic diet (KD), originally designed physiological effects that occur as a result of fasting, such as ketosis, in order to mimic its protective outcomes. Fasting, which has been used for centuries as an unproven method for controlling seizure disorders, primarily results in increased levels of ketone bodies and causes the body begin using stored fat as the primary energy source as opposed to glucose (Thiele, 2003, Ziegler, et al., 2003). The KD was developed as way to mimic both the increase in ketone bodies and shift of metabolic utilization without depriving patients of essential nutrients and energy. The regime requires a shift in the ratio of fat: carbohydrate consumption from roughly 1:2 to 4:1 (Rho, et al., 2004, Thiele, 2003). Many versions of the ketogenic diet have been examined for efficacy in attenuating seizure, of which the program shown to have the best efficacy is a reduced calorie regime combined with the increased fat: carbohydrate ratio (Rho, et al., 2004).

Although, the mechanism of the KD is not fully understood; it has been shown to increase antioxidant enzymes, such as glutathione peroxidase (GPx), as well as upregulate specialized mitochondrial uncoupling proteins (fig. 3) thereby reducing ROS and oxidative damage by supporting the endogenous antioxidant system as well as decreasing the amount of ROS actually produced. (Sullivan, et al., 2004). The reduction of ROS and oxidative damage, coupled with preferential utilization of an efficient energy source (ketone bodies), in neuronal tissue could explain how this treatment proves to be an effective therapy for epileptic seizure, however, the rigorous constraints on caloric intake has been a stumbling block for its wide spread implementation, most patients opting for an alternative pharmacological treatment (Rho, et al., 2004)

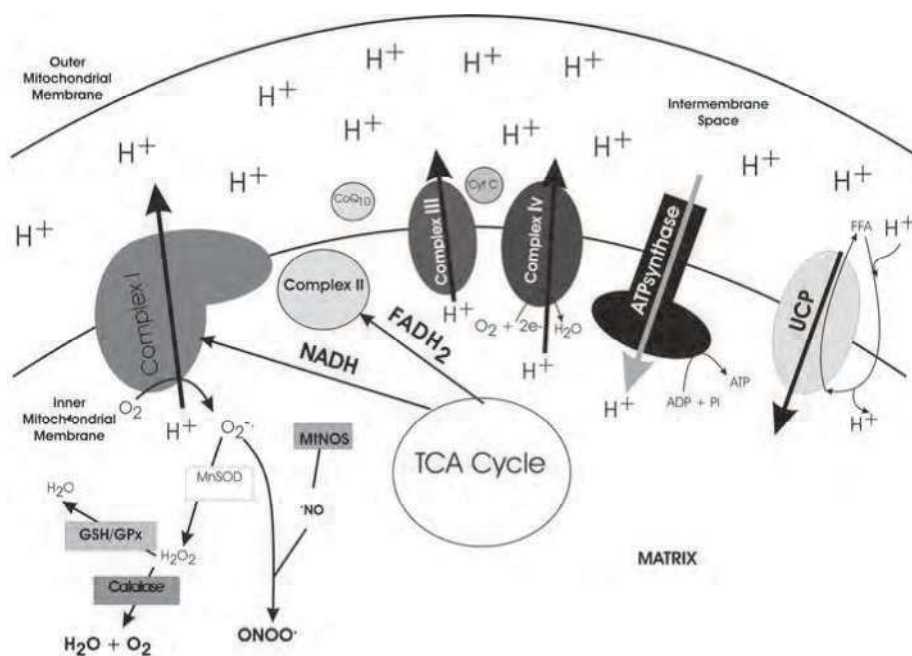


Fig. 3. This is a schematic of the mitochondrial electron transport chain (ETC). Electrons are donated by reducing agents (NADH and FADH₂) which flow down the ETC causing the pumping of protons (H⁺) into the intermembrane space, thereby creating a proton gradient (separation of charge). It is by this mechanism that the cell is able to produce energy in the form of ATP by utilizing this proton gradient to phosphorylate ADP to ATP via Complex V (ATP synthase). Oxidative phosphorylation produces reactive oxygen species (ROS) as a normal byproduct of physiological function. ROS is mostly produced at Complex I; however it can be produced at Complex III/IV (via the same mechanism pictured at Complex I) as well. Endogenous antioxidant systems such as GSH and MnSOD prevent the formation of peroxynitrite (ONOO⁻) which can lead to mitochondrial and cellular damage/dysfunction. Uncoupling Proteins (UCP) can dissipate the proton gradient by translocating protons from the intermembrane space to the mitochondrial matrix in response to activation by free fatty acids (FFA).

9. Conclusion

Most of the AEDs discussed in this chapter are effective treatments for a wide range of seizure disorders; however each has their distinct pros and cons. There are some AEDs that are better to administer in a pediatric setting, where as others will work better for patients with chronic seizure disorders. Most, if not all, depend on the proper dosing to achieve their optimum treatment effect. Only when the therapeutic dose is surpassed is there an increased risk of potential side effects that may terminate that avenue of treatment options.

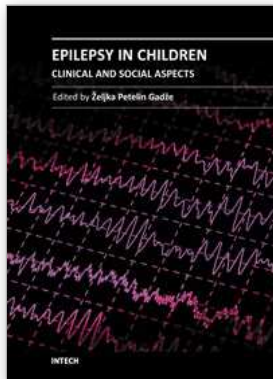
It has been shown that there is a correlation between seizure development and oxidative damage, which in turn causes a state of hyper-excitability causing the initiation of future seizures due to the increased sensitivity to excitation. It has become apparent that mitochondria are intimately involved in this mechanism due to the involvement of mitochondrial superoxide dismutase (MnSOD) in the attenuation of seizure induced oxidative damage (Liang and Patel, 2004). The attenuation of this oxidative damage could lead to a decrease in the initiation of prolonged seizures and further oxidative damage, thereby ending this vicious cycle. Therapeutic interventions and AEDs designed to attenuate oxidative damage and decrease the total level of excitation to reduce the incidence of seizure and the amount of subsequent damage will be the most beneficial. AEDs that provide neuroprotection, in addition to their anticonvulsant properties, are currently in use and should be further studied so that other treatments may be developed with neuroprotection, not only attenuation of SE, in mind.

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Epilepsy is a neurological condition that accompanies mankind probably since its inception. About 400 years before Christ, the disease was already known by Hippocrates, who wrote the book "On The Sacred Disease". Classically, epilepsy has been defined as a chronic condition characterized by an enduring propensity to generate seizures, which are paroxysmal occurring episodes of abnormal excessive or synchronous neuronal activity in the brain. Out of all brain disorders, epilepsy is the one that offers a unique opportunity to understand normal brain functions as derived from excessive dysfunction of neuronal circuits, because the symptoms of epileptic seizures are not the result of usual loss of function that accompanies many disease that affect the brain. I am therefore extremely honoured to present this book. The 15 very interesting chapters of the book cover various fields in epileptology – they encompass the etiology and pathogenesis of the disease, clinical presentation with special attention to the epileptic syndromes of childhood, principles of medical management, surgical approaches, as well as social aspects of the disease.

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