In general, the key mechanisms that mediate transformation of a group of normally functioning neurons into epileptic neurons are complex and are likely to vary with causative factors responsible for the initiation of epileptogenesis. The membrane lipid peroxidation due to increased oxidative stress or deficiency of antioxidant defenses appears to be causally involved in some forms of epilepsies, and decreases in antioxidant enzyme activities may cause the increased risk of an idiosyncratic drug reaction (Kurekci, 1995). Human post-traumatic epilepsy is one example where the neuronal membrane lipid peroxidation caused by iron released from the extravasated haemoglobin plays an important causative role in epileptogenesis. The model of focal electrographic seizures induced by intracortical injection of FeCl$_3$ in the rat is of great interest as it models post-traumatic human epilepsy. Epileptogenesis in this model involves lipid peroxidation events significantly. In the present thesis this model has been studied as an example of lipid peroxidation mediated epilepsies. The data reported herein are from the ferric chloride (FeCl$_3$)-induced cortical epilepsy in the rat.

Iron-induced epilepsy in rodents is an animal model of post-traumatic epilepsy of humans. Head trauma (closed head injuries) in humans may result in a haemorrhage in the brain. Extravasation of the blood leads to the release of haemoglobin. The Fe$^{2+}$/Fe$^{3+}$ ions liberated from the lysed haemoglobin may interact with the tissue structures. The post-traumatic epilepsy in humans is believed to result from an acquired lesion(s) due to interaction between iron ions released from the haemoglobin and nerve cells. The Fe$^{2+}$/Fe$^{3+}$ ions interact with the neural tissue membranes and catalyze the formation of oxygen free radicals i.e. O$_2^-$, OH$^-$ etc. The action of these radicals with neural membrane lipids leads to the formation of lipid peroxides, thereby changing the lipid environment and composition of the membrane. Recent laboratory data have shown that alterations in the membrane lipid environment render the nerve cells hyperexcitable. Iron compounds such as FeCl$_3$ or FeCl$_2$ injected into the cortex of experimental animals produce focal epilepsy. Intracortical injection of Fe$^{2+}$ or Fe$^{3+}$ in rat cerebral cortex produces an electrophysiologically active chronic epileptiform focus. The iron-induced model so produced is considered to be an important reliable model for post-traumatic epilepsy.
Ever since this model was first discovered in 1978, it continues to be of almost uninterrupted research interest and investigations have provided data that have helped to understand the underlying mechanisms of post-traumatic epilepsy and new therapeutic strategies. Even in some clinical cases the use of vitamin E as adjunct therapy has been found useful.

Since lipid peroxidation appears to mediate the development of post-traumatic epilepsy, therapeutic measures that can counter lipid peroxidation might be useful in preventing the development of epileptogenic focus. A few investigations have tried to find out whether pretreatment with the anti-lipidperoxidant agent α-tocopherol can prevent the development of iron-induced epilepsy in animal models. The results have indicated that the pretreatment of experimental animals with α-tocopherol (vitamin E) prevents the development of iron-induced epileptiform activity. One study has however, also shown that pretreatment with α-tocopherol does not completely abolish iron-induced epilepsy but only significantly delays its onset. In the present work, an attempt has been made to study whether antiperoxidant drugs in general may prevent the development of iron-induced epilepsy by studying the effects of a number of antiperoxidant drugs. Furthermore, since in clinical practice the treatment will be given only after epilepsy has appeared in a head trauma case, it would be more meaningful to investigate whether pharmacological administration of antiperoxidant agents such as α-tocopherol is effective when administered somewhat subsequent to seizure onset. Therefore, in the present study antioxidant drugs were administered at day 3 after creation of the iron-induced epileptogenic focus.

Therefore, the main aim of the present work was to study the effect of anti-lipid-peroxidation agents on iron-induced epilepsy in the rat brain model to find out whether antiperoxidant drugs can suppress the iron-induced experimental epileptiform activity. In this work, effects of α-tocopherol, verapamil, and flunarizine have been studied besides ethosuximide and sodium valproate. The latter four substances are known to be Ca$^{2+}$ channel blockers (Ca$^{2+}$ antagonists). Ca$^{2+}$ antagonists are known to have a wide range of antioxidant potencies and in that respect several of them are potent anti lipid peroxidants. In epileptogenesis, Ca$^{2+}$ channel blockers have also been found useful because Ca$^{2+}$ influx through T-type Ca$^{2+}$ channels is an important event underlying the development of paroxysmal depolarization shift (PDS) in
epileptogenesis. Some epilepsies are due to $\text{Ca}^{2+}$ channel defects resulting from mutations in $\text{Ca}^{2+}$ channel genes.

To assess the anti-lipidperoxidant activity of the drugs studied ($\alpha$-tocopherol, verapamil, flunarizine, ethosuximide and sodium valproate) the following parameters were studied: lipid peroxidation (TBA products and 4-HNE) and glutathione peroxidase. These two parameters were selected because it has been earlier indicated that in the iron-induced epileptogenic focus lipid peroxidation increases and is maintained at a high level together with a low level of glutathione peroxidase activity. Even in clinical cases of childhood seizures the glutathione peroxidase deficiency was suggested to be a cause of seizures and antiepileptic drugs were found to elevate plasma glutathione peroxidase levels. Valproic acid is known to increase glutathione peroxidase activity in epileptic children. Therefore, alterations in the glutathione peroxidase levels are of considerable significance. In the present study, the development and occurrence of epileptic activity was monitored electrocorticographically. The data showed that these drugs when administered subsequent to the onset of seizure activity were effective against iron-induced epilepsy. The data further indicated that the antioxidant drugs like vitamin E besides being useful as adjunct or add on therapeutic agents might also be important as antiepileptic / anticonvulsant agents in their own right. The results also provide information about the involvement of $\text{Ca}^{2+}$ channels and lipid peroxidation in the development of iron-induced epilepsy.
2. INTRODUCTION

In the ancient writings, epilepsy has been considered as a sacred disease. It has been shrouded in mystery and fear. It is one of the most enigmatic ones faced by man. It is generally defined as a chronic disorder, or a group of disorders, characterised by seizures that usually recur unpredictably. The term epilepsy is derived from the Greek word meaning ‘to seize upon’. It was first described by Hughlings Jackson in the nineteenth century as an intermittent derangement of the nervous system due to a sudden, excessive, disorderly activity of a group or groups of cerebral neurones. Now we know that epilepsy should better be designed as a syndrome, that is a collection of symptoms, some of which are secondary to other brain derangements and some of which are seemingly primary (Fisher, 1989). An epileptic neuron is a hyperexcitable cell, i.e. a cell which has acquired a tendency to produce electrical discharges considerably higher than the normal. The difference between a normal and an epileptic neuron is that the latter responds with a burst to a stimulus that would produce a single action potential in the former (Reid and Sypert, 1980). Epileptic seizures may result either from excessive excitation or from a reduced inhibition of a group of epileptic neurons. The electroencephalogram (EEG) is used to study epilepsy and is a time varying record of electrical potential differences at paired points of the scalp (Fisher, 1989).

The EEG hallmarks of focal and generalised epilepsy, both in animal models and in human epilepsy, are the ictal or seizure discharge and the inter-ictal spike discharge (ID). In some forms of epilepsy, seizure discharges originate electrically and anatomically from the site of spike discharges. In acute focal epilepsy, during the ID, thousands of neurons in the focus synchronously undergo unusually large depolarisation shifts (DS) superimposed on which is a burst of action potentials. The DS is followed by a hyperpolarising potential (the post-DS HP) and neuronal inhibition. During ID, at the site of the focus, extracellular levels of Ca\(^{2+}\) decrease presumably because of exit of K\(^+\) from, and entry of Ca\(^{2+}\) into, neuronal processes. When a seizure develops post-DSSH become smaller and disappears and is replaced by a depolarisation. These series of events occur synchronously in the population of neurons within the focus and the EEG shows after discharges (AD) after several successive IDs. The AD becomes longer with each ID and progresses into a seizure
(Dichter and Ayala, 1987). The PDS result from the alterations in the membrane’s ionic conductances that may arise from changes in the synaptic neurotransmission. However, the shifts can also result from the non-synaptic alterations in the intrinsic properties of the nerve cell membrane (Witte et al., 1987). The nerve cell membrane properties are of crucial importance in the generation of hyperexcitability because the excitability properties reside in the membrane.

A group of nerve cells is generally responsible for hyperexcitability and hypersynchronisity seen in epileptic activity. This means that an epileptic neuron expresses its increased activity only in an epileptic neuronal aggregate. An excessive electrical epileptic discharge from a group of neurons in one or the other region of the brain (Rodin, 1987) can produce changes in the state of consciousness of the subject and also convulsions.

Many questions arise on the mechanism of epilepsy at the cellular level. Animal models and clinical cases are being studied in an attempt to answer the many questions associated with epilepsy (Trottier et al., 1983; Fischer and Alger, 1984; Massotti et al., 1984; Turski et al., 1985; Griffith et al., 1987; Speckmann et al., 1989). Of several experimental epilepsy models, iron-induced experimental model is widely used as it mimics histological and electrophysiological features of human post-traumatic epilepsy (Willmore et al., 1977; Willmore et al., 1978 a and b; Ciuffi et al., 1991; Dakin, 1993).

Post-traumatic epilepsy is characterised by epileptic seizures due to brain damage secondary to head injury. Although it has been known since the days of Hippocrates, the pathogenesis of post-traumatic epilepsy remains unclear. Rosen and Frumin (1979) reported that haemoglobin could induce epileptic discharges just as iron ion does. This evidence suggests that the epileptogenic effect of haemoglobin may be dependent on ionic iron released from haemoglobin (Mori et al., 1990). Head trauma initiates a sequence of responses that includes altered blood flow and vasoregulation, disruption of the blood-brain barrier, increases in intracranial pressure, focal or diffuse ischemia, haemorrhage, inflammation, necrosis and disruption of fibre tracts. Extravasation of blood is followed by haemolysis and deposition of heme-containing compounds into the neuropil initiating a sequence of univalent redox reactions and generating various free radical species, including superoxides, hydroxyl radicals, peroxide and perferryl ions (Willmore, 1990). The
interaction of these free radicals with neural membranes may render the latter hyperexcitable that is epileptogenic. In animal models, post-traumatic epilepsy can be induced by ferrous or ferric chloride injection. Intracortical injection of ferrous or ferric chloride solution in the rat has been found to produce acute and chronic epileptic activity (Willmore, 1978a). The epileptic activity so induced has been shown to last for 3 months or more (Willmore, 1978b). Subsequently electrophysiological findings (Reid and Sypert, 1980) have contributed to the understanding of the initial features of iron-induced epilepsy. Peroxidation of lipids in the cortical neuronal membranes has been seen to occur at the iron-injection site concomitantly with the appearance of epileptic activity (Triggs and Willmore, 1984). Not much is known however, concerning the whole process (Moriwaki et al., 1992). Electrocorticographic characteristics (Willmore et al., 1978a and b), extracellular activity of cortical neurons (Reid and Sypert, 1980) and their enzymatic activities have been reported in acute iron-induced epilepsy. There have also been reports concerning alterations in the electrocorticograms (ECoGs) in chronic iron-induced epilepsy (Moriwaki et al., 1990). Histologically intracortical injection of iron salts causes focal edema, necrosis, gliosis etc. (Willmore et al., 1986). The effect of various metal ions on neuronal membrane fluidity was examined by electron spin resonance and it was found that ferric ions decreased the membrane fluidity (Ohba et al., 1994). The interaction of several critical factors leads to the abnormal synchronizations (McNamara, 1992). Changes also take place in the amino acid levels during the iron-induced epileptiform activity (Shiota et al., 1989).

Mechanisms underlying the induction of epilepsy by iron remains to be precisely elucidated. Biochemical and physiological consequences of iron-tissue interaction (Halliwell and Gutteridge, 1986; Triggs and Willmore, 1984) are of considerable interest. The inorganic iron salts hematin and haemoprotein stimulate peroxidation of lipids of the membranes of microsomes and mitochondria and also produce changes in sulphide bonds (Hoekstra, 1975; Orlowski and Karkowski, 1976).

Since membrane enzymes require molecular integrity of the membrane lipid compartment, physiological consequences of oxidative stress, membrane lipid peroxidation and other lipid derangements merit consideration (Nelson and Delgado-Escueta, 1986; Pellmar, 1986 and 1987; Nakaya et al., 1987; Tretter and Adam-Vizi, 1996; Fowler, 1997). Data derived from the experiments conducted on a variety of
tissues have indicated that peroxidation of the membrane lipids would affect properties like membrane fluidity, functional activity and efficiency of membrane enzymes leading to impairment of signal generation as for example in guinea pig hippocampus in vitro (Pellmar, 1986). This and other studies, provide the evidence that significant changes in electrophysiological properties of the neuron can result from the effect of lipid peroxidation on Na⁺,K⁺-ATPase, channel molecules, neurotransmitter receptors etc. (Fong and McNamee, 1986; Housley et al., 1986). Recently it has been shown that under certain conditions, lipid peroxidation can decrease synaptic efficacy and tend to support the theories which implicate membrane lipid peroxidation in the mechanism of iron-epileptogenesis. The EEG of an iron-injected rat showed isolated spikes and spike and wave complexes near the injection site approximately for 30 days or more after the injection. This was seen on the contralateral side too (Moriwaki et al., 1990). A secondary focus thus develops at the contralateral site. This secondary epileptogenesis may be defined as the sum of the series of events that result in development of epileptiform hyper-irritability in an initially ganglionic region synaptically related to an area in which a chronic epileptogenic lesion has been experimentally produced or as in humans occurring naturally. This secondary site may become independent of the primary focus (Morrell, 1982). In iron-induced epilepsy, partial section of corpus callosum resulted in a decrease in the number of rats showing spike activity on the side of secondary focus (Moriwaki et al., 1985). When iron staining was done in brain sections of iron-induced epileptic rats, the iron was stained brown coloured in the vicinity of the injection area. The iron stained areas developed on the opposite side through the corpus callosum (Mizukawa et al., 1991) but it is generally believed that secondary focus was unaltered by the chemical agent that produced the chemical lesion (Mayersdorf and Schmidt, 1982).

2.1. ANTIOXIDANT DEFENCE SYSTEMS

Active oxygen (superoxide, peroxide and hydroxyl radicals) and other free radicals occur naturally as intermediates in oxidative metabolism. There are many enzymes and free radical scavengers present in normal tissue to maintain these compounds within non-toxic levels (Pellmar, 1986). The imbalance between oxidative
reactions and protective mechanisms in the cells is therefore the basis of a number of
diseases (Ursini, 1985). The majority of these effects of oxygen radicals are
associated with a measurable formation of lipid peroxides (Viani, 1991).

In order to counteract the oxyradicals and to minimise their damage, aerobic
cells have developed an elaborate system of antioxidant mechanisms. An antioxidant
is any substance that when present at a low concentration as compared to that of an
oxidizable substrate prevents or inhibits the oxidation of that substrate (Halliwell and
Gutteridge, 1989). Endogenous antioxidants include both non-enzymatic and
enzymatic compounds (Ursini, 1985). α-Tocopherol, ascorbic acid, glutathione,
selenium, ubiquinones and uric acid are the important non-enzymatic antioxidants. Of
these α-tocopherol and ubiquinones are the lipid soluble antioxidants while ascorbic
acid, glutathione, selenium and uric acid are the aqueous antioxidants. The enzymes
superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase
constitute the important enzymatic antioxidants (Niki, 1987).

In cells, there is a steady state of the formation of oxyradicals, met by a similar
rate of their elimination by antioxidants. An imbalance in this equilibrium towards
pro-oxidants is termed as oxidative stress (Sies, 1985). The accumulated oxidative
damage as a result of oxidative stress has been implicated in many pathophysiological
states, one of the important being epileptiform activity.

2.1.1. Glutathione peroxidase

Glutathione peroxidase (GPx) using glutathione as a co-substrate and selenium
as a metallic cofactor reduces intracellular formation of hydrogen peroxide and free
radicals. Only GPx is able to do so (Maker, 1983). GPx levels sometimes are seen to
dercrease when lipid peroxidation increases (Singh and Pathak, 1990; Hothersall et al.,
1981; Yalcin et al., 1986; Tamura et al., 1998). GPx utilises the reducing power of
glutathione and contains selenium. It reacts with a wide variety of lipid
suggest that GPx exerts its protective effect by preventing free radical mediated
peroxidation and it inhibits the formation of hydroxyl radicals by NADH oxidase
(Leibovitz, 1980).
2.2. **ANTIEPILEPTIC DRUGS**

In common usage an antiepileptic drug (AED) is a drug when administered over a prolonged period will decrease the incidence or severity of seizures occurring in patients with epilepsy. Any drug that on single dose administration leads to a long term abolition of seizures could also be accepted as AED (MacDonald and Meldrum, 1995). The drugs examined in this study were chosen on their ability to act as antioxidants, free radical scavenger or as calcium channel antagonist or both. The drugs studied were:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tocopherol</td>
<td>An antioxidant</td>
</tr>
<tr>
<td>Verapamil</td>
<td>Calcium channel blockers</td>
</tr>
<tr>
<td>Flunarizine</td>
<td>showing antioxidant</td>
</tr>
<tr>
<td></td>
<td>Valproate showing antioxidant</td>
</tr>
<tr>
<td>Ethosuximide</td>
<td>Calcium channel blocker</td>
</tr>
<tr>
<td></td>
<td>with suspected antioxidant activity</td>
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</table>

2.2.1. **Antioxidants**

These are chemical molecules which scavenge free radicals and prevent the generation of oxidative stress in cell.

2.2.1.1. **α-Tocopherol**

Vitamin E is the generic name given to a group of 8 lipid soluble compounds that are derivatives of tocopherol and tocotrienol. Of them α-tocopherol has the greatest biological activity and accounts for more than 90% of vitamin E in the tissues (Southam et al., 1991). Therefore, the term vitamin E and α-tocopherol are used interchangeably (Halliwell and Gutteridge, 1989). It is the major lipid soluble chain breaking antioxidant (Dean and Cheeseman, 1987) in mammalian
cell membranes (Molennar et al., 1980). \( \alpha \)-tocopherol in the membrane can break the free radical chain efficiently to inhibit the lipid peroxidation (Takaneka et al., 1991) (Figure 1). Vitamin E can react directly with a variety of oxyradicals including the peroxyl radical \( \text{ROO}^\cdot \), \( \text{CCl}_3 \) and \( \text{OH} \) (McCay, 1985; Burton et al., 1985), superoxide radical \( \text{O}_2^- \) (Fukuzawa and Gebicki, 1983) and singlet oxygen (Sies and Murphy, 1991). The fully methylated aromatic ring and chromane moiety ensure near maximal stabilisation of the tocopheroxyl radical formed in the rate limiting reaction of \( \alpha \)-tocopherol with oxyradical (Burton and Ingold, 1989).

The kinetic study suggests that one molecule of vitamin E scavenges two molecules of peroxyl radicals (Yamamoto et al., 1984; Niki et al., 1984; Burton and Ingold, 1981; Niki, 1987). \( \alpha \)-tocopherol is absorbed via the lymphatic pathway and transported in association with chylomicrons. In plasma, \( \alpha \)-tocopherol is found in all lipoprotein fractions, but mostly associated with apo B-containing lipoproteins in man. In rats, 50% of \( \alpha \)-tocopherol is bound to high density lipoproteins (HDL). After intestinal absorption and transport with chylomicrons tocopherol is mostly transferred to parenchymal cells of the liver where most of the fat soluble vitamin are stored. Little vitamin E is stored in the non-parenchymal cells (endothelial, stellate and Kupffer cells). Tocopherol is secreted in association with very low density lipoprotein (VLDL) from the liver.

Some recent studies have, however, shown that \( \gamma \)-tocopherol which is the major form of vitamin E in the diet is more effective than \( \alpha \)-tocopherol with respect to inhibition of lipid peroxidation (Wolf, 1997). Vitamin E is the key antioxidant for the inhibition of lipid peroxidation damage (Pryor, 1971; Tappel, 1970). For optimum nutrition it is required in relatively large amounts and it is non-toxic at very high levels (Tappel et al., 1973). It is important for the maintenance of normal neurological
Fig. 1: Sites of action of vitamin E (α-tocopherol)
structure and function (Goss Sampson and Muller, 1987; Sokol, 1988). It plays an important role in the maintenance of the integrity and stability of neuronal membranes. The nervous tissue appears to be exquisitely sensitive to vitamin E deficiency as evidenced by the occurrence of vitamin E deficiency - associated neuropathological lesions such as degeneration of the axons of the gracile and cuneate nuclei in the brain stem, posterior columns of the spinal cord and in the peripheral nerves with a selective loss of myelinated fibres (Nelson, 1987). The mechanism of neurological lesions in vitamin E deficiency has not been established. However, various studies (Nelson, 1981 and 1987; Southam et al., 1991; Meydani et al., 1988) suggest that neurological sequel of vitamin E deficiency results from the antioxidant properties of the vitamin. It was seen that when α-tocopherol was exhausted in ghost cell membranes, peroxidation of membrane lipids occurred.

α-tocopherol has been studied as an AED for iron-induced experimental epilepsy, in animal models. The anticonvulsant effects of α-tocopherol have also been tested in four other animal seizure models - the Metrazol threshold model (MET), the maximal electroshock model (MES) and the kindling model. Vitamin E failed to antagonise the seizures in the MES, MET or the kindling model. It was however able to significantly delay the onset of electrographic seizures in the intracerebral iron chloride model, pointing to the fact that lipid peroxidation could be involved in this particular model of epilepsy (Levy et al., 1990). However, in this study by Levy et al. (1990), iron-induced electrographic seizures were not found to be completely prevented by α-tocopherol, only their onset was delayed by it. In another study on post ischemic reperfusion, lipid peroxidation in rat cerebral cortex, was reduced considerably on injecting α-tocopherol (Vanella et al., 1992).

The striking histopathological changes caused by iron injection into rat isocortex and subsequent chronic epileptogenesis (Willmore and Rubin, 1981 and 1984) were found to be prevented by the pretreatment of animals with α-tocopherol (Rubin and Willmore, 1980). Pretreatment with α-tocopherol also protected cat spinal cord (Anderson and Means, 1983; Saunders et al., 1987) and also prevented compression-induced edema in rat brain (Yoshida et al., 1983). Acute treatment with parenteral α-tocopherol as the alcohol, could alter the initiation of lipid peroxidation with intracerebral iron injection sites. In experiments in which epilepsy was induced
by injecting iron salts into the amygdala of rats pre-treated with \(\alpha\)-tocopherol, it was observed that levels of lipid peroxidation were significantly reduced (Triggs and Willmore, 1994.). \(\alpha\)-Tocopherol pretreatment also prevented the occurrence of convulsive seizures in a significant number of iron injected animals. Lipid peroxidation measured in the dissected hippocampus was significantly increased in untreated rats developing iron-induced seizures, but in rats pre-treated with \(\alpha\)-tocopherol it decreased. Moreover \(\alpha\)-tocopherol failed to prevent bicuculline induced seizures (Willmore et al., 1986). Sodium metavanadate administration to rats intraperitoneally, for some consecutive days, was found to enhance lipid peroxidation in the rat brain. Administration of \(\alpha\)-tocopherol has also been found to decrease the vanadium-stimulated lipid peroxidation in the rat brain (Haider and Fakhri, 1991).

It is clear from the above discussion that so far only the effects of pretreatment with \(\alpha\)-tocopherol have been studied. Since in clinical cases pharmacological treatment will be given after epilepsy symptoms have appeared it would be more relevant to find out if treatment with \(\alpha\)-tocopherol would be efficacious in suppressing the epileptiform activity if administered after iron-induced seizure onset. Therefore, the objective of the present work was to study the seizure-suppressing effects of \(\alpha\)-tocopherol in animals that have already developed iron-induced epileptiform activity. Following injection of iron, electrographic seizures begin to appear as early as one hour after the injection (Levy et al., 1990) and continue thereafter for considerably long time (Moriwaki et al., 1990, 1992). In the present study, treatment with drugs was begun three days after the creation of the iron-induced lesion. Furthermore, to have an insight into the mechanism which may mediate antiepileptic effect of \(\alpha\)-tocopherol, changes in the activity of glutathione peroxidase, and in the levels of lipid peroxidation were assessed during treatment with \(\alpha\)-tocopherol in the ipsilateral iron-induced epileptogenic focus as well as in the contralateral focus. The data derived from measurements of these parameters were expected to show if \(\alpha\)-tocopherol prevented epileptic activity by anti-lipidperoxidant mechanisms.
2.2.2. Calcium channels and their antagonists

Antiepileptic drugs (AEDs) are known to have effects on calcium channels (Macdonald and McLean, 1986). Calcium entry into neurons has been shown to be through multiple voltage-dependent calcium channels (Nowycky et al., 1985). Calcium channels have been classified into different types (Fox et al., 1987; Tsien et al., 1988; Hess et al., 1986) (Figure 2a). Based on their voltage dependence and pharmacological profiles six types of voltage-gated calcium channels appear to exist: L, N, T, P, Q and R types (Figure 2b). The best characterised mechanism of calcium influx is brought about by voltage-operated calcium channels (VOCs). These are calcium selected pores that are normally activated for milliseconds during action potentials and can be found in neurons, muscle cells and endocrine cells.

Receptor operated calcium ion channels are directly gated by a ligand and are found in excitable cells (which respond to excitatory neurotransmitters e.g. acetylcholine, glutamate, serotonin) as well as in non excitable cells responding to agonists such as ATP, histamines. These channels are usually referred to as non-selective cation channels. They are not nearly as selective for calcium as VOCs.

Second messenger operated calcium permeable ion channels share many properties with receptor operated calcium ion channels. The main difference is that former are gated from the intracellular cytosolic side by G proteins or second messengers secondary to receptor stimulation. Second messenger operated calcium permeable ion channels are found in olfactory neurons and photoreceptor cells where cyclic nucleotides (cAMP and cGMP) mediate the activation of cation channels (Penner et al., 1993).

Calcium release activated Ca\(^{2+}\) channels are gated by an unknown mechanism following depletion of intracellular calcium stores presumably the endoplasmic reticulum stores. The luminal Ca\(^{2+}\) content is registered by a putative Ca\(^{2+}\) sensor which transmits a signal to the plasma membrane channels. This could be a direct coupling or via a generation of messengers (Penner et al., 1993). It is documented that calcium influx is involved in epileptogenesis (Pumain et al., 1983; Heinemann and Hamon, 1986) which is probably involving voltage operated calcium channels (Ullal, 1994) i.e. T-type Ca\(^{2+}\) channels. Some epilepsies have been thought to be ion channel disorders resulting from defects in their (ion channel) genes (Bievert, 1998).
Fig. 2a: Schematic representation of the various types of Ca\textsuperscript{2+} conducting ion channels and their gating mode.

Fig. 2b: Types of voltage operated channels
example, a neonatal human epilepsy is due to mutation and consequent loss of function of potassium channels, a form of human partial epilepsy is due to defects in acetylcholine receptor; and some genetic epilepsies of mice are due to defects in \( \text{Ca}^{2+} \) channels. \( \text{Ca}^{2+} \) antagonists however, do not depress non-epileptic neuronal activity. A synchronous massive discharge of cortical neurons is the principal phenomenon observed in epilepsy. A consistent electroencephalographic feature in these patients is the interictal 'spike': a large extracellular field potential, associated with paroxysmal depolarising shifts (PDS) in neuronal membrane potentials that trigger action potentials. Intrinsic bursting may be dependent on calcium influx into neurons. This PDS may also require synchronisation of a population of neurons through synaptic mediation. Experimental findings indicate that calcium and calcium dependent currents participate in the generation of these events. Consequently neuronal PDSs were found to be depressed by organic calcium channel blockers and so were seizure discharges in neuronal populations. The extracellular calcium concentration of identified neurons of snails steeply decreased with the commencement of paroxysmal depolarization and started to increase again when the PDS had reached its plateau level. It has been shown that an influx of calcium ions takes place during PDS (Lucke et al., 1990).

Indirect evidence for the role of neuronal calcium flux in epileptiform activity also includes the observation that some anticonvulsants inhibit calcium calmodulin-stimulated protein phosphorylation in the presynaptic terminal. Furthermore, phenytoin inhibits brain synaptosome calcium uptake and has been recently shown to inhibit release of intracellular calcium in snail neurons stimulated with pentylenetetrazole (Meyer et al., 1986).

Excitatory amino acid receptors, particularly of the N-methyl-D-aspartate (NMDA) type, are involved in the genesis and development of the various experimental seizures (Croucher et al., 1982; Czuczwar and Meldrum, 1982; Meldrum et al., 1983) and in the establishment of long-term potentiation and kindling. Activation of excitatory amino acid receptors have been shown to induce calcium fluxes across the neuronal membrane (MacDermott et al., 1986; Mody and Heinemann, 1986) and electrophysiological studies have indicated that entry of calcium into the neurons might represent a potential substrate for epileptogenesis. In fact, epileptiform bursts are often associated with influx of calcium ions into nerve
cells and a decrease in the extracellular concentration of calcium precedes the onset of seizures in many experimental models of epilepsy (Heinemann et al., 1981; Pumain et al., 1986). Moreover, the blockers of voltage dependent calcium channels display anticonvulsant activity in various models of experimental convulsions (Ashton and Wauquier, 1979) and in humans (Overweg et al., 1984). Both seizures and the decrease in extracellular calcium were prevented by flunarizine, a blocker of voltage dependent calcium channels. These findings suggest that voltage dependent calcium channels might contribute to influx of calcium ions and seizures caused by excitatory amino acids.

Calcium currents contribute to epileptogenesis by underlying bursting in pacemaker cells and by enhancing post synaptic excitatory responses in dendrites and soma of nerve cells and by providing post-burst re-excitation (Heinemann and Hamon, 1986). Calcium currents carry calcium from the extracellular to the intracellular space. Experimental findings indicate that a calcium inward current and the calcium dependent membrane currents participate in the generation of focal epileptic activity. PDS was found to be depressed by the intracellular and extracellular applications of the organic calcium channel blockers like verapamil and flunarizine (Speckmann et al., 1989).

On investigating effects of the voltage dependent calcium channel blockers it was found that verapamil - an L-type calcium channel blocker, showed a dose dependent decrease of dopamine release. Treatment with ω-conotoxin, an N-type channel blocker decreased 50% basal dopamine release. Treatment with flunarizine, a T-type channel blocker did not affect the dopamine release. From this it appears that treatment of L and N type VSCC in rat striatum suppress basal dopamine release but not T type blocker suggesting different mechanism of action (Kato et al., 1992).

While sharing the ability to impede calcium entry into the heart muscle cell (myocyte) via the voltage dependent slow calcium channel (Triggle and Janis, 1987), antagonists may also possess ‘cytoprotective’ properties independent of calcium channel blockade (Kloner and Braunwald, 1987; Tatani et al., 1988). A previous investigation by Janero et al. (1988) demonstrated that some calcium antagonists reduce the susceptibility of cardiac membrane phospholipid to free radical induced peroxidation. In purified rat brain synaptosomes, mitochondria and cultured fetal mouse spinal cord neurons, calcium was observed to enhance markedly the lipid
peroxidation mediated damage (Braughler et al., 1985). Lipid peroxidation increased
the permeability of membranes to calcium through the release of fatty acids and their
oxidation products (Watras et al., 1984; Philipson and Ward, 1985; Siesjo and
Weiloch, 1985). Thus, lipid peroxidation and calcium are intimately and inseparably
involved in the pathogenesis of cell injury by oxygen free radicals (Singh et al.,
1985).

The interactions between lipid peroxidation and calcium in mediating damage
to central nervous system membranes, have been examined in several in vitro
systems. Calcium was found to markedly enhance lipid peroxidation-induced
disruption of membrane functions. The findings suggest that calcium and lipid
peroxidation cannot be considered as separate entities in the pathophysiology of CNS
trauma.

Depending on their mechanism of action, anticonvulsant drugs in clinical use
may be divided into three groups: those drugs which facilitate GABAergic
neurotransmission; those which block neuronal ion channels; and those whose
mechanism of action is unresolved. The example of the first type is barbiturates and
the benzodiazepines. Ethosuximide blocks voltage-operated calcium channels
specially those which mediate calcium currents in thalamic neurons. Of those drugs in
which the mechanism of action is unknown, sodium valproate is the prime example.
An antagonistic action at the NMDA subtype of glutamate receptor might also be a
possibility.

Calcium channel antagonists can be of many chemical classes, some of the
major ones being the dihydropyridines e.g. nifedipine and nitrendipine, the
benzodiazepines e.g. diltiazem, the phenylalkylamines e.g. verapamil, the
diphenylpiperazine e.g. flunarizine and cinnarizine, sodium valproate, a fatty acid and
the succinimides e.g. ethosuximide.

These attributes of calcium channel blockers i.e., their ability to reduce influx
of calcium into the cells and also to reduce lipid peroxidation, were exploited in this
study. Since antiepileptic effects of Ca^{2+} channel blockers may be mediated by their
anti-lipidperoxidative property, effects of a few Ca^{2+} channel blockers have been
studied in the present work to see if iron- induced epileptiform activity is suppressed
by Ca^{2+} channel blockers. The seizure-suppressing effects of verapamil,
ethosuximide, flunarizine and sodium valproate have been investigated in this work.
2.2.2.1. **Verapamil**

Verapamil is a phenyl alkylamine and a derivative of papaverine. It inhibits the slow calcium flux. Its action can only be assessed when the channel is open. It directly blocks influx of calcium through the slowly inactivating voltage sensitive L-type channels. The drug suppresses firing of the SA node and also produces vasodilation in smooth muscles that accounts for its use as an antihypertensive agent. It slows AV conduction and thus ventricular response. Verapamil is also useful for patients with arterial fibrillation. It inhibits the entry of calcium in cardiac, vascular, gastrointestinal and brain tissues. Verapamil exerts an antiepileptic effect on neurons. This effect is assumed to depend on the blockade of transmembrane calcium flux during epileptic discharge (Wiemann et al., 1996).

Organic calcium antagonists have been reported to abolish epileptic neuronal discharges elicited by pentylenetetrazole (PTZ) and penicillin. Verapamil (40-80 μM) reduced the amplitude, duration and the frequency of appearance of the paroxysmal depolarization shifts induced by bicuculline, which it does so by blocking GABAergic synaptic inhibition. Therefore, calcium currents are also involved in bicuculline PDS (Straub et al., 1990). It was seen that prolonged verapamil perfusions gradually reduced the rate and duration of burst discharges in bicuculline-induced epileptiform discharges. Action potential amplitude was decreased and action potential duration increased by verapamil treatment (Aicardi and Schwartzkroin, 1990).

The calcium channel antagonist verapamil dissolved in artificial cerebrospinal fluid, when applied by intraventricular push-pull, reduced focal interictal epileptiform discharges (FIED) in amplitude and in the frequency of occurrence. In some experiments, the suppression of seizure activity was preceded by a transient
enhancement. After termination of the drug perfusion FIEDs were often re-established. Control experiments revealed that perfusion with drug free cerebrospinal fluid did not change FIEDS. In experiments without epileptic activity, cortical evoked potentials elicited by stimulation of the sciatic nerve tended to increase with perfusion of the calcium antagonist. As a whole the systemic administration of the calcium antagonist verapamil depressed FIED and exerted an inverse effect on synchronized non-epileptic neuronal activity (Walden et al., 1985). Administering verapamil reduced the effect of excessive calcium influx following CNS lesions (Darlington and Smith, 1992; Wong and Rahwan, 1989).

Antiepileptic effect of verapamil was manifested in a reduced frequency and amplitude of spike discharges, decreased power of epileptic foci and less frequent appearance of seizure (ictal) discharges (Karpova et al., 1987). There was a reduction of PDS induced by pentylenetetrazole (PTZ) in neocortical as well as archicortical neurons (Bingmann et al., 1988).

Some studies have shown that verapamil inhibits lipid peroxidation. It inhibits iron dependent lipid peroxidation, by its antioxidant properties (Reddy et al., 1996; Sugawara et al., 1996). The drug afforded protection against ischemia / reperfusion-induced lipid peroxidation and reduced the release of serum glutamate pyruvate transaminase (SGPT). This beneficial effect of verapamil occurs only in rats sensitized to oxidative injury suggesting that the calcium channel blocker protects against oxygen free radical attack (Stein et al., 1993). Even in mitochondrial ischemia followed by reperfusion, oxygen free radicals were formed and membrane degradation resulted from stimulated phospholipase activity due to formation of lipid peroxides. Pretreatment by verapamil kept the total phospholipid content at control levels. It also maintains the level of glutathione at control levels (Kajiyama et al., 1987). Thus, verapamil also exerted an antioxidant effect and was shown to do so by Mak and Weglicki, (1994). Using arachidonic acid metabolism, free radical mediated damage could be diminished significantly by antioxidant effects of verapamil (Roth et al., 1991). The drug verapamil inhibited free radical generation (Prabha et al., 1990). The superoxide radical formation accelerated by calcium ions was prevented by verapamil and superoxide dismutase (Nakashima et al., 1990). Another study showed that both verapamil and cinnarizine prevented the formation and accumulation of lipid peroxidation in tissues and normalised the basic components of the system of
antioxidant protection (Savchenko et al., 1996). Verapamil also reduced the oxidation of human LDL in vitro (Napoli et al., 1996). Lipid peroxidation induced in isolated rat hepatocytes by FeCl₃ was associated with an increase in the cytosolic free calcium. Cytosolic calcium accumulation was completely prevented by vitamin E. Preincubation with verapamil also inhibited the elevation of cytosolic calcium (Albano et al., 1991). Verapamil inhibited in vitro microsomal lipid peroxidation by inhibiting phospholipase A₂ (al Bayati and Stohs, 1991). The above mentioned studies thus show that verapamil has significant antioxidant action.

There have been some reports which state that verapamil has a poor blood-brain penetration (Wauquier, 1985) or that it does not pass the blood-brain barrier or reach effective concentration in the cerebrospinal fluid after oral administration (Kohling et al., 1993). But a number of reports substantiate the fact that verapamil indeed penetrates the blood-brain-barrier and reduces epileptiform activity by both acting as a calcium channel antagonist and as an inhibitor of lipid peroxidation (Vanella et al., 1992; Vezzani 1988, Meyer et al., 1986; Kamal et al., 1990; Darlington and Smith, 1992; Stein et al., 1993; Reddy et al., 1996; Sugawara et al., 1996).

### 2.2.2.2. Flunarizine

Flunarizine \{(E)-1-[bis (4-fluorophenyl)-methyl]4-(3-phenyl-2-propenyl) piperazine\} is a difluorinated piperazine derivative (Cousin et al., 1983). It
is well absorbed and reaches peak levels within 2-4 hrs after oral administration. It is highly lipophilic and it readily crosses the blood-brain barrier. It is structurally related to cinnarizine. Flunarizine is removed slowly from the body. Its plasma concentration in patients with epilepsy appeared lower than that in healthy people taking the same dose (Kataoka et al., 1988; Yamaji et al., 1987). This may be the result of hepatic enzyme induction (Binnie et al., 1985). It is a well tolerated medication that is effective against partial seizures in some patients. It has a long half life and because of this once a day dosing is possible. No significant to minimal adverse effect has been reported in clinical trials. The effects reported have been varied but have included body weight gain, drowsiness, headaches and a change in mood and alertness. In none of the clinical trials was flunarizine discontinued because of toxicity (Alvinz et al., 1989; Strarreveld et al., 1989; Keene et al., 1989; Bebin and Bleck, 1994).

It is a selective calcium antagonist, i.e. it blocks the entry of excess calcium ions into cells preventing calcium overload which damages cells. It does not interfere with normal cellular calcium. It inhibits contraction of vascular smooth muscle mediated by extracellular calcium. It also dilates the cerebral arteries in vitro. It is effective in numerous animal models of hypoxia/anoxia. It has a protective effect against ischemia caused by acute thrombosis. Flunarizine also has antihistaminic properties (Bebin and Bleck, 1994, Homes et al., 1984) and is effective against vertigo (Heykants et al., 1979; Overweg et al., 1984).

Flunarizine reduced PTZ induced paroxysmal depolarizations of neocortical neurons (Bingmann et al., 1988; Rodger and Pleuvry, 1993). It potentiates strongly the potency of AEDs like valproate and significantly raises threshold for electroconvulsions on its own (Czuczwar et al., 1992). The anticonvulsant effects of small doses of nifadipine and also of small doses of carbamazepine and phenytoin were enhanced by flunarizine.

Flunarizine has been widely acknowledged as a calcium channel antagonist in a number of tissues including cardiac, vascular smooth muscle, endothelial cells, erythrocytes and brain. Extensive investigations using non-neuronal tissues have demonstrated that flunarizine shows predominant specificity for L-type voltage dependent calcium channels (VDCCs). In the brain, blockage of VDCCs by flunarizine can affect epileptic seizures but the precise brain calcium channel type inhibited by flunarizine is not clear. At 30 μmoles, flunarizine selectively blocked a
T-type calcium current having no effect on the L-type current in a mouse neuroblastoma cell line. Flunarizine was found to inhibit both, calcium-dependent and -independent glutamate release from synaptosomes and cultured cerebellar neurons (Cousin et al., 1993). The drug also reduced depolarization-induced presynaptic calcium accumulation, possibly in part by blocking calcium influx through a channel similar if not identical to the N-type channel. This pharmacological action of flunarizine may be one of its mechanism of protection following cerebral ischemia (Cohan et al., 1991).

In a therapy resistant epileptic population with partial complex seizures with or without secondary generalisation, addition of flunarizine to existing therapy was accompanied by a significant reduction in complex partial and tonic-clonic seizures (Overweg et al., 1984).

Flunarizine is an effective prophylactic treatment for common or classic migraine in children and adults (Todd and Benfield, 1989). When comparative studies between different AEDs were carried out it was seen that in genetically epilepsy prone rats (GEPR) anticonvulsant activity of calcium channel antagonist flunarizine was most potent (De Sarro et al., 1990). Flunarizine has anti-dopaminergic activity related to its neuroprotective actions (Finnegan et al., 1993). It reduced the viability of dopamine rich human neuroblastoma cells in vitro (Mena et al., 1995). It also has a potent inhibitory effect on tetrodotoxin (TTX) sensitive calcium current, suggesting that abnormal excess excitation of TTX sensitive calcium channels may be one of the trigger factors generating epilepsy (Takahashi et al., 1992).

There are some reports showing inhibitory action of flunarizine on lipid peroxidation. Although free radical formation and lipid peroxidation have been implicated in photoreceptor degeneration following continuous light exposure, recent work has shown that flunarizine ameliorates light-induced retinal degeneration and that elevated intracellular calcium may indeed play a role in enhancing the levels of lipid peroxidation (Edward et al., 1991). On examining the effect of various metal ions on neuronal membrane fluidity, it was found that ferric ions decreased the membrane fluidity while ferrous ions increased it at each high concentration. Flunarizine elevated it at each high concentration. It also shows antioxidant protection in diabetic angiopathy in rats by inhibition of lipid peroxidation and prostanoid synthesis. The effect was more pronounced by its additional blocking effect of
abnormal calcium flux. Thus flunarizine appears to show free radical scavenging property (Somova et al., 1989).

Flunarizine and verapamil both showed significant inhibitory effect on the peroxidation of rat liver microsomes in the presence of iron ions and ascorbic acid (Aruoma et al., 1991). The inhibitory effects of calcium antagonist on mitochondrial swelling induced by lipid peroxidation on arachidonic acid in the rat brain in vitro was studied by Takei et al., (1994). Their results revealed that mitochondrial swelling and lipid peroxidation induced by FeSO₄ and ascorbic acid were inhibited by all the calcium antagonists tested, the order of inhibition being flunarizine > nicardipine > efonidipine > nimodipine > nifedipine, suggesting that these calcium antagonist tested have antiperoxidant activities resulting in protection against mitochondrial damage. They hypothesised that flunarizine, possibly, could directly interact with mitochondrial membrane and stabilise it. Similar studies on radical scavenging action of flunarizine in rat brain mitochondria were done by Kubo et al., (1984). They showed that although flunarizine was shown to be active in any system, the most pronounced effect was elicited in mitochondria where it was three times greater than that of tocopherol. These studies suggested that free radical scavenging or antioxidant activity of flunarizine may play a role in its brain protective action.

In the present study, flunarizine's effects have been studied on the iron-induced epilepsy in rat. This antioxidant action and its calcium channel antagonist properties were further investigated in this study.

2.2.2.3. Ethosuximide

Ethosuximide (3 ethyl-3-methyl 2,5 pyrroldinedione) has been widely used for treatment of absence seizures for over 30 years. It is a chiral drug,

![ethosuximide](image)

containing one asymmetric carbon atom and is used clinically as a racemate. Several reports concerned with the metabolism of ethosuximide have been published.
Ethosuximide has been demonstrated to reduce T-current in the thalamic relay neurons and in primary afferent neurons at therapeutically relevant concentration. It has been proposed that T-calcium currents are important pacemaker currents in thalamic relay neurons and that these currents may be responsible in part for the 3 Hz rhythm seen in the EEG of patients with generalised absence seizures. Reduction of T-calcium current by ethosuximide then would disrupt the slow rhythmic firing of thalamic neurons and disrupt the spike and wave discharge (Macdonald and Meldrum, 1995). Its effect on thalamic nucleus is well documented. Electrophysiological studies in cats have shown that sleep spindles which develop into spike and wave discharges can be diminished by the administration of ethosuximide, thus reducing the activity of the reticular thalamic nucleus as well as a decrease in the incidence of epileptic spike and wave discharges (Steriade, 1993).

Ethosuximide is one of the means of treatment of minor epilepsy but its mechanism of action remains to be elucidated. It is generally assumed that the probable mechanism of action of ethosuximide consists of lowering the calcium transport (Toreva et al., 1991).

In a study, spontaneous generalised epileptiform discharges were elicited in rodent thalamocortical slices which showed spike and wave, and generalised tonic-clonic seizure disorders. The drug ethosuximide reduced these bursts by blocking T-current in thalamic neurons (Zhang et al., 1996). The effects of ethosuximide on calcium channels were also studied on dorsal root ganglion neurons from one day old rats using the patch clamp technique. Bath application of ethosuximide induced dose dependent and reversible suppression of calcium currents without affecting their time course. Substantial differences between the effects of ethosuximide on the low threshold and high threshold (T and L-type) calcium currents were observed. It reduced the T-current with greater potency than the L current (Kostyuk et al., 1992).

As is already known, ethosuximide prevents absence (petit mal) epilepsy (Aicardi, 1986; Browne et al., 1975) and it affects the low threshold calcium current (LTCC) (Coulter et al., 1989a and1989b). This current is mediated by a voltage sensitive calcium channel present at a high density in thalamic neurons. The effect of ethosuximide on the LTCC is not matched by other anticonvulsants. It diminishes the low threshold calcium spike while leaving the tonic firing pattern unaffected and its effects on low threshold calcium current might selectively alter the dynamics of slow
bursting in thalamic cells (Lytton and Sejnowski, 1992). Ethosuximide is commonly used to treat seizures (petit mal) as it delays the onset of the seizure discharge as well as delays the increase in seizure duration (Stringer et al., 1994). Ethosuximide possibly decreases high frequency repetitive firing of action potentials by reducing a low threshold calcium current (T-channel) (Macdonald and Kelly, 1995).

The effects of AEDs, on seizures of the tremor rat showed profiles similar to those observed in human absence seizures, with paroxysmal bursts of spike wave complexes. These were inhibited by ethosuximide 100-200 mg/kg i.p. and valproate 1000 mg kg i.p. Ethosuximide, which prevents the spread of seizure activity through cortical pathways, shows an increase in the levels of glucose utilisation when administered to genetic absence epilepsy rats (GAER), as the seizure activity may be an additional energy consuming process (Nehlig et al., 1993). When it was given to acoustically stimulated epileptic rats and withdrawn an increase in glutamate level was seen. This increase can be correlated with the increased seizure susceptibility (Voiculescu et al., 1994). Currents evoked by the application of GABA to acutely dissociated thalamic neurons were analysed by voltage clamp techniques and it was seen that the drug ethosuximide did not occlude the effects of bicuculline on GABA responses. Ethosuximide in high concentration (1-10 mM) reduced GABA levels to a lesser extent but blocked calcium currents of thalamic neurons (Coulter, 1990a and b).

Effects of ethosuximide on different types of induced epilepsies (experimental epilepsies) have also been reported. Only high doses of ethosuximide can suppress clonic seizures in picrotoxin-induced seizures. Tonic-clonic seizures probably have model specific sensitivity to ethosuximide because this drug completely suppressed PTZ induced tonic-clonic seizures but had no effect on kainic acid-induced tonic-clonic seizures (Veliskova et al., 1993). Valproate and ethosuximide administered intraperitoneally, both showed dose-dependent antiepileptic activity synergistically towards PTZ-induced myoclonic and tonic-clonic seizures (Musolino et al., 1991). It has been suggested that ethosuximide depresses the transmission of repetitively evoked responses through an increased release of the transmitter not compensated by a similar increase in the rate of its replenishment leading to a progressive depletion of the transmitter itself. Considering results of their experiments, Pelligrini et al. (1989) have stated that ethosuximide induces its action on either the cortex, thalamus or mid-
brain reticular formation. No reasonable explanation, of the peculiar efficacy of ethosuximide in suppressing the spike and wave discharge, has yet been presented.

Ethosuximide with its calcium channel blocking properties was scrutinised in this study to see if it brought about any changes in lipid peroxidation mediated epilepsy i.e. the iron-induced experimental epilepsy.

2.2.2.4. Valproic acid

Valproic acid is a branch chained fatty acid, structurally unrelated to any other AED due to its lack of a nitrogen molecule or a heterocyclic moiety. It has a relatively short half-life (9-16 hrs), diurnal variation in absorption and clearance, non linear protein binding and large diurnal fluctuations of the binding. It is metabolized primarily by β-oxidation (and also other pathways). 1-3% of the administered dose is excreted unchanged in the urine but most of the parent drug and several of its metabolites undergo conjugation with glucoronic acid (Dickinson et al., 1989). Sodium valproate has been in clinical use for the treatment of epilepsy in Great Britain since 1973 and in the United States since 1978. It has been shown to be an effective antiepileptic drug in a wide variety of seizure types but clinically its major use to date has been in generalised seizures. It is particularly effective in photosensitive epilepsy and in myoclonus. Its efficacy and safety in adults and children for the treatment of generalised seizures (absence, tonic-clonic, myoclonic), partial seizures, simple and complex secondarily generalised seizures have been well documented. Adverse effects associated with the drug are gastrointestinal disturbances (nausea, vomiting, dyspepsia); although the use of enteric-coated formulations has reduced the incidence of abdominal discomfort. Weight gain, tremors and transient hair loss are also reported. Importantly, valproate has minimal neurological adverse effects (sedation, ataxia, impairment of cognitive functions) (Davis et al., 1994). On the whole, most adverse reactions to sodium valproate are mild and reversible, but with experience the drug’s rare idiosyncratic effects are
becoming apparent particularly hepatotoxicity and teratogenicity (Rimmer and Richens, 1985).

The precise mechanism of action of valproic acid, like that of many other AED is unknown. Much attention has been focused on its effect on GABA, one of the principle inhibitory neurotransmitters in the CNS. One of the earliest studies in rats suggested that the antiepileptic effect of valproic acid was the result of elevated brain GABA levels, resulting from the inhibition of the cytosolic enzyme GABA-transaminase (GABA-T) (Godin et al., 1969). Valproate also enhances the function of GABAergic synapses at the level of the GABA-coupled chloride channel. Thus, GABAergic transmission appears to play an important role in the molecular mechanism involved in the antiepileptic action of sodium valproate (Concas et al., 1991).

Valproic acid is also known to activate the enzyme glutamic acid decarboxylase (GAD) (Loscher, 1981; Phillips and Fowler, 1982; Taberner et al., 1980), a major enzyme in GABA synthesis. The drug, however, strongly inhibits the enzyme succinic semialdehyde dehydrogenase (SSADH), an enzyme involved in the GABA degradation pathway (Van der Laan et al., 1979, Zeise, 1991). For example, it was shown that valproic acid increased GABA levels via inhibition of GABA-T in mouse synaptosomes (Loscher, 1981; Loscher and Vetter, 1985). The GABA-T reduction observed in synaptosomes may be due to a higher susceptibility of nerve terminal GABA-T to the inhibitory effects of valproic acid. Valproic acid was also found to selectively enhance post-synaptic GABA responses (Gent and Phillips, 1980; MacDonald and Bergey, 1979), although the clinical evidence of this effect is not known (Davis et al., 1994). In experiments on the model of generalised PTZ-induced epileptic activity in male Wistar rats, the efficacy of the combination of the drugs influencing different mechanisms of epileptogenesis was studied. It was seen that sodium valproate enhanced GABAergic processes and when used in combination with ryodipine produced more marked antiepileptic effect than each of these drugs alone (Kryzhanovskii et al., 1992a).

Valproate also induces certain cytochrome P-450 monooxygenase activities and decreases glutathione-S-transferase activity (Rogeirs et al., 1995). It also demonstrates antimanic properties (Chen et al., 1994). The evidence also exists which suggests that valproic acid reduces neurotransmission mediated by excitatory amino acids.
acids such as aspartic acid, glutamic acid, γ-hydroxybutyric acid (GHB) (Chapman et al., 1982; Vayer et al., 1988; Whittle and Turner, 1978).

Valproic acid was also found to reduce sustained repetitive firing (SRF) through its influence on sodium and potassium conductances (Davis et al., 1994). When effects of clinically used anticonvulsant drugs were studied on high frequency sustained repetitive firing (SRF) of action potentials, it was seen that valproate limited the SRF (Macdonald et al., 1985). The effectiveness of valproate and ethosuximide among other clinical anticonvulsant drugs was evaluated in the quaking mouse model of epilepsy. In this model tonic-clonic seizures are easily elicited by handling. Valproate was found to be an effective anticonvulsant agent in this model as it reduced epileptic bursts (Taylor et al., 1985). Effect of valproate was also studied on L-(1-ML) glutamine and L-(1-ML) glutamate metabolism in isolated human kidney-cortex tubules. Valproate markedly stimulated glutamine removal as well as the formation of ammonia, pyruvate, lactate and alanine but it inhibited glucose synthesis (Martin et al., 1990). Effects of sodium valproate were studied on neocortical pyramidal cells (layer II/III) of the rat in vitro by intracellular recording (Zeise et al., 1991). It, in a dose related manner, suppressed the characteristic transient depolarization induced by NMDA. Higher concentrations of valproate also reduced L-glutamate responses. At these concentrations it increased the duration of orthodromically evoked inhibitory post-synaptic potentials and reduced repetitive spike firing induced by depolarising currents. These results suggest that an essential mode of action for the anticonvulsant valproic acid is the attenuation of NMDA receptor mediated excitation.

Rats that had limbic seizures induced by pilocarpine were given valproic acid and ethosuximide and it was seen that valproic acid was most effective against spontaneous recurrent seizures (Leite and Cavalheiro, 1995). Valproic acid possibly decreases high frequency repetitive firing of action potentials by enhancing sodium channel inactivation and by reducing the low threshold T-channel (Macdonald and Kelly, 1995). At different doses valproate delayed the onset and increase of seizure duration (Stringer, 1994). Valproate showed different degrees of reduction in the duration of the tonic phase before reaching doses that produce neurological symptoms in rats (Gonzalez, 1995).
Histological effects of valproic acid have also been assessed in some studies. Effective doses of valproate (200 mg/kg) applied in rats chronically evoked the first morphological changes in the brain hemispheres after nine months of drug administration. Structural abnormalities of the brain tissue consisted of disseminated non-specific neuronal and patchy nerve cell loss. The neuronal lesions were localised predominantly in the third and fifth layers of the neocortex and in the pyramidal cell layer of the hippocampus (Sobaniec et al, 1992).

Some AEDs may alter trace elements' metabolism and free radical scavenging enzyme activities in humans and experimental animals. It was seen that valproate-treated children had an increase in glutathione peroxidase activity which may bring lipid peroxidation down (Kurekci et al., 1995). Valproate is also known to influence lipid peroxidation regulating system without itself being an antioxidant (Nikushkin et al., 1993).

Valproic acid has demonstrated antiepileptic effects in animal models of absence, partial, and generalised seizures induced by a variety of chemical, electrical or sensory stimuli. It was active against generalised (absence, clonic, tonic-clonic) seizures induced by a variety of chemical convulsants. This drug also prevented quinolinic acid-induced seizures, and suppressed behavioural alterations in a rat model of human complex partial seizures (Vezzani et al., 1986).

In the present study it was considered of interest to investigate the effects of valproate on iron-induced epilepsy model.