"TO EVALUATE AND COMPARE THE ANTIEPILEPTIC EFFECT OF CALCIUM CHANNEL BLOCKERS AND THEIR ABILITY TO POTENTIATE THE ANTIEPILEPTIC EFFECT OF EXISTING ANTIEPILEPTIC DRUGS IN RAT MODELS"

By

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BLDE University Vijayapur, Karnataka

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of the requirements for the degree of

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IN

PHARMACOLOGY

Under the guidance of

DR.AKRAM A. NAIKAWADI

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Date:

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LIST OF ABBREVIATIONS

AEDs	Antiepileptic drugs
AMPA	alpha amino-2,3-dihydroisoxazolepropanoic-5-methyl-3-oxo-4 acid
ANOVA	One way analysis of variance
BFNE	Benign familial neonatal epilepsy
CYP	Cytochrome P-450
DRE	Drug resistant epilepsy
EME	Early myoclonic encephalopathy
FS+	Febrile seizures plus
GABA	γ-aminobutyric acid
GTCS	Generalized-tonic-clonic seizure
HLE	Hind limb tonic extension
i.p.	Intraperitoneal
ILAE	International League Against Epilepsy
MEI	Myoclonic epilepsy in infancy
MES	Maximal Electroshock
NMDA	N-methyl-D-aspartate receptor
PDS	Paroxysmal depolarization shift
PTZ	Pentylenetetrazol
SEM	Standard error of mean
SV2A	Synaptic vesicle glycoprotein 2A
TLE	Temporal lobe epilepsy

ABSTRACT

Background: The currently available antiepileptic drugs have a low therapeutic index, and provide satisfactory seizure control in only 60-70% of patients. Calcium channel blocker has shown potentials of a useful add-on drug for the existing antiepileptic drugs.

Materials and Methods: Antiepileptic potential of calcium channel blockers (nifedipine and verapamil) was evaluated in MES and PTZ models of epilepsy in comparison and combination with phenytoin (25 mg/kg) and sodium valproate (250 mg/kg) in albino wistar rats; half the dose was used when calcium channel blockers (nifedipine and verapamil) was combined with either phenytoin or sodium valproate. The time taken before the onset of clonic convulsions (latency), the duration of clonic convulsions, the percentage of seizure protection and percentage mortality were recorded.

Results: Calcium channel blockers (nifedipine and verapamil) were found to reduce the durations of tonic extensor phase, duration of convulsion in a statistically significant way in the both MES and PTZ model; and while used in combination with phenytoin and/or sodium valproate, the results were statistically significant than both the drugs given individually. In both these group statistically significant increased percentage epilepsy protection and decrease in percentage mortality was noted when compared to control groups ($p < 0.05$).

Conclusion: Calcium channel blockers (nifedipine and verapamil) have shown potency as an individual antiepileptic drug as well as a useful add-on therapy with standard antiepileptic drugs like phenytoin and sodium valproate in both the models of epilepsy.

KEYWORDS: Epilepsy, Nifedipine, Verapamil, Antiepileptic drugs, MES, PTZ

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INTRODUCTION

Epilepsy is one of the most common afflictions of man with a prevalence of approximately 0.3-0.5% in different population.[1] It has important medical, social, psychological consequences.[2]

The causes of seizures are extremely diverse and include the full range of neurological diseases from infections to neoplasm, head injury. Heredity has proved to be a predominant factor in some subgroups.[3,4] Even though it was recognised as early as 2000 B.C. new concepts about its pathogenesis, etiology and treatment are brought out almost every year.[5] In the past the treatment of the epilepsy was based on superstitious religious beliefs and ignorance. But the present day concept of treatment of epilepsy is very much different from what it was earlier.[6] The therapeutic objective of the treatment of epilepsy is complete seizure control without excessive side effects. The investigators who have worked on epilepsy have used various chemicals for treating epilepsy, starting from bromides. [which provides the first rational treatment for patients suffering from epilepsy.] to recent newer antiepileptics.[7] Inspite of the vast number of drugs introduced for the treatment of epilepsy, there is still a need for an ideal antiepileptic agent with properties like broad spectrum activity, rapid onset of action, least side effects, good oral bioavailability and low cost.[8]

Clinically used calcium channel blockers act by blocking L-type and T-type of calcium channels which are present in various tissues including brain and they are commonly prescribed for treatment of various clinical conditions such as hypertension, angina and cardiac arrhythmias.[9] Many tissues outside the CVS are functionally

dependent upon the influx of extracellular calcium through various channels on the cell membrane. It is clear that Calcium Channel Blockers may be of value in treating many pathological states where over activity of calcium channels is apparent. Beneficial effects of calcium channel blockers have been found in various central nervous system disorders like nerve repair and regeneration migraine etc.[10] Epilepsy is one such disorder & experiments conducted on individual neurons shows that calcium ions play a decisive role in the origin of epileptic activity. Calcium influx is critical to the process of repetitive synchronus firing of neurons which leads to epilepsy. Voltage dependent calcium currents are believed to play multiple roles in epileptic events; they may contribute to the depolarization shift seen in focal epilepsy They may become active during the transition to seizures & spread of seizure activity & they may be particularly important in some forms of partial generalised epilepsy.[11]

Three types of calcium channels [T, N & L] that are modulated in a different manner by different drugs exist in neurons.[12] The organic calcium channel antagonists are also classified in 4 structurally different classes. Each of them has a variable level of activity in different excitable tissues[13] and therefore, they may also have differences in their major pharmacological actions and the clinical uses. It has rightly been suggested that calcium channel blockers have distinct subgroups that can be used as a basis for drug design and prediction of clinical efficacy at an early stage in drug development.[14]

Keeping this in view, a comparative evaluation of antiepileptic effect of clinically used calcium channel blockers namely nifedipine and verapamil used to evaluate their ability to potentiate the antiepileptic effect of existing drugs has been made using experimental models of epilepsy.

OBJECTIVES

Aims and Objectives of the Study

1. To evaluate the antiepileptic potential of calcium channel blockers and to compare with existing antiepileptic drugs.

2. To evaluate the ability of the calcium channel blockers to potentiate the anti-epileptic effect of existing anti-epileptic drugs.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

A. EPILEPSY

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- **Pentylenetetrazol (PTZ)**
- **Phenytoin**
- **Sodium valproate**
- **Calcium channel blockers**
    a. **Nifedipine**
    b. **Verapamil**

REVIEW OF LITERATURE

A. EPILEPSY

Origin

Mid 16th century: from French *épilepsie*, or via late Latin from Greek *epilēpsia*, from *epilambanein*‘seize, attack’, from *epi* ‘upon’ + *lambanein* ‘take hold of’.

Epilepsy is a group of neurological disorders characterized by epileptic seizures.[15] Epileptic seizures are episodes that can vary from brief and nearly undetectable to long periods of vigorous shaking.[16]

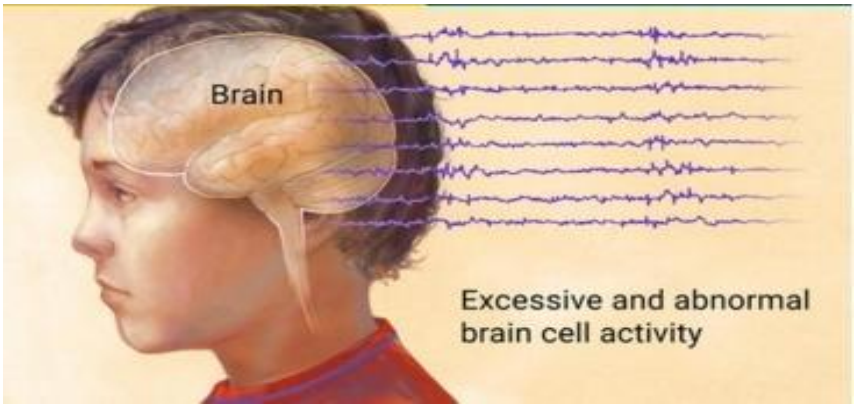


Figure 1: Epilepsy

Definition

In 2005, a Task Force of the International League Against Epilepsy (ILAE) formulated conceptual definitions of seizure and epilepsy:[17]

"An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain."

"Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures, and by the neurobiologic, cognitive, psychological, and social consequences of this condition. The definition of epilepsy requires the occurrence of at least one epileptic seizure."

In order to bring the practical (operational) clinical definition of epilepsy into concordance with how epileptologists think about epilepsy, the ILAE Task Force recommends broadening the definition of epilepsy to include the circumstances:[18]

Epilepsy is a disease of the brain defined by any of the following conditions

1. At least two unprovoked (or reflex) seizures occurring >24 h apart
2. One unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years
3. Diagnosis of an epilepsy syndrome Epilepsy is considered to be resolved for individuals who had an age-dependent epilepsy syndrome but are now past the applicable age or those who have remained seizure-free for the last 10 years, with no seizure medicines for the last 5 years.

Aetiology

Epilepsy is a major neurological disorder, the symptoms of which are preventable and controllable to some extent and which has deep clinical, psycho–socio–demographic and economic implications that vary across different regions of the world and are associated with varied incidence, prevalence and mortality.[19-21] Despite substantial advances in

epileptology for identification of newer syndromes, for example, molecular and structural patterns and so on, a wide gap still exists that stems mainly from epilepsy's extremely heterogeneous and complex set of risk factors. The major factors that may influence the risk of epilepsy described in **Figure 2**.

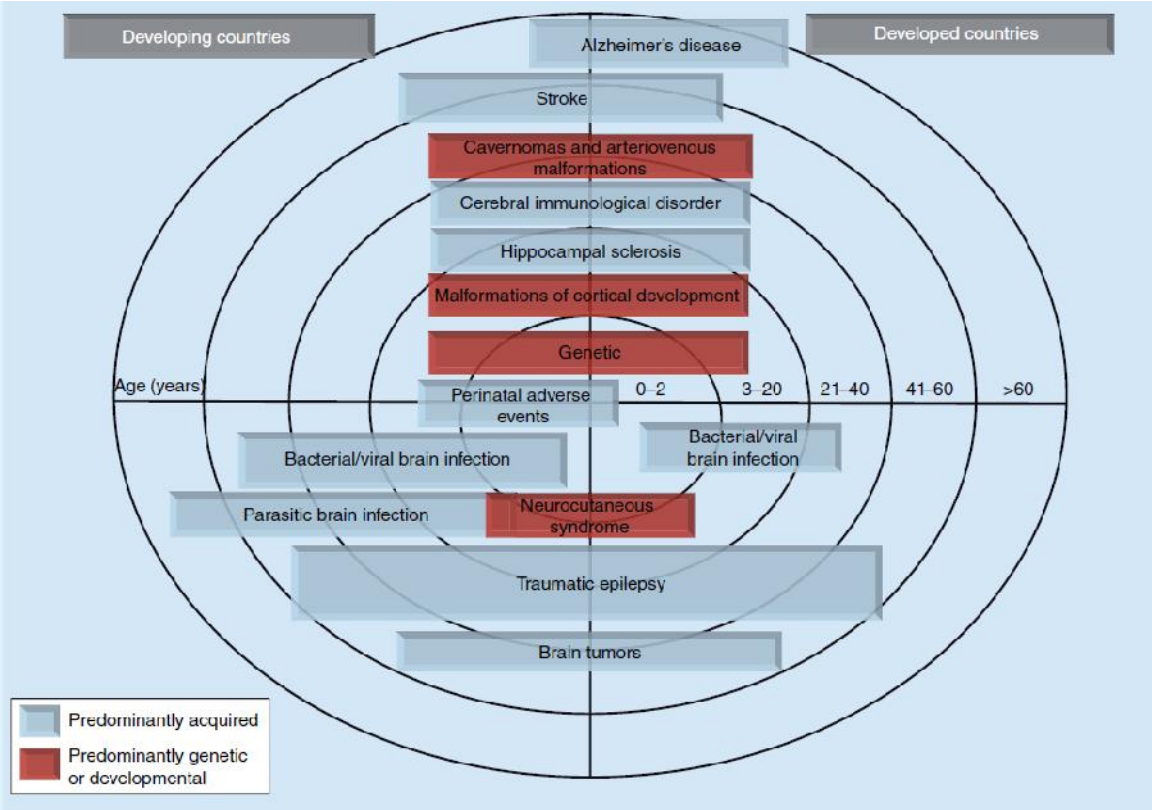


Figure 2: Distribution of epilepsy etiologies by age[22]

It is evident that epilepsy is a complex and heterogeneous disorder with a long list of risk factors. Figure 1 revealed epilepsy etiologies and highlighted the main risk-defining parameters as far as possible (injury severity, low grade tumors especially of temporal or rolandic localization, early onset, genetic origin of AD and so on), which might promote timely identification of a suitable target population (people at risk of developing epilepsy). Variable significance of individual etiologies (stroke, perinatal trauma,

infections in low–middle income countries [LMIC]) and presence of cofactors (other diseases or clinical symptoms) may lead to region-specific epilepsy frequencies. A direct role of alcohol in epilepsy onset is less convincing. The role of onchocerciasis in epilepsy onset remains possible but subject to further research. Many gene mutations representing rarer epilepsy forms have been discovered but are of limited day-to-day clinical value. Particular attention should be paid to cerebral malaria, AD and limbic encephalitis. Populations in many countries (e.g., North Europe) are aged/aging and this may lead to a consequential increase in AD-related epilepsy in the near future. Perinatal factors and infections do represent significant causes of epilepsy in many pockets of LMICs but these conclusions are drawn in the absence of sufficient population-based studies and their independent effect as epilepsy triggers has most probably been over highlighted; for example, the independent significance of perinatal trauma as a causal factor may get diluted by the presence of family history of epilepsy. They also overshadow other more relevant etiologies such as stroke and cerebral malaria in these populations. Moreover, the wealth of information available to develop epileptogenic drugs, for instance inhibitors of the mTOR pathway, may modify the course of tuberous sclerosis and consequentially the risk of epilepsy onset. Most drugs of today are seizure-controllers. This should go hand-in-hand with the need to develop specific epilepsy prevention methods that may constitute newer research avenues. Finally, there is a need to develop LMIC-specific ‘ideal epilepsy epidemiology monitoring criteria’ since the guidelines that currently exist do not fit the challenges that an epidemiological investigation on epilepsy in these countries may have. Problems in these countries are different and may need different solutions.

Epilepsy is one of the most common serious neurological disorders[9 23] affecting about 39 million people as of 2015.[24] It affects 1% of the population by age 20 and 3% of the population by age 75.[25] It is more common in males than females with the overall difference being small.[26,27] Most of those with the disorder (80%) are in the developing world.[28]

The estimated prevalence of active epilepsy (as of 2012) is in the range 3-10 per 1,000, with active epilepsy defined as someone with epilepsy who has had a least one unprovoked seizure in the last five years.[27,29] Epilepsy begins each year in 40-70 per 100,000 in developed countries and 80-140 per 100,000 in developing countries.[28] Poverty is a risk and includes both being from a poor country and being poor relative to others within one's country.[26] In the developed world epilepsy most commonly starts either in the young or in the old.[26] In the developing world its onset is more common in older children and young adults due to the higher rates of trauma and infectious diseases.[26] In developed countries the number of cases a year has decreased in children and increased among the elderly between the 1970s and 2003.[29] This has been attributed partly to better survival following strokes in the elderly.[27]

Pathophysiology

PDSs are cellular events in which rapidly repetitive action potentials are not followed by the usual refractory period, thereby generating a prolonged membrane depolarization (which is more prolonged than typically occurs in response to normal excitatory postsynaptic potentials [EPSPs]). The paroxysmal depolarization shift (PDS) is the pathophysiological cellular phenomenon that underlies all types of epileptic seizures (**Figure 3**) and interictal epileptiform electroencephalography (EEG) abnormalities

("spikes").[30,31] An interictal spike is caused by PDSs in large numbers of neurons that are synchronized such that each involved neuron generates one PDS at the same time. An electroclinical seizure occurs when large numbers of neurons in one or more brain regions are repeatedly generating PDSs, in sustained repetitive firing with synchronization.

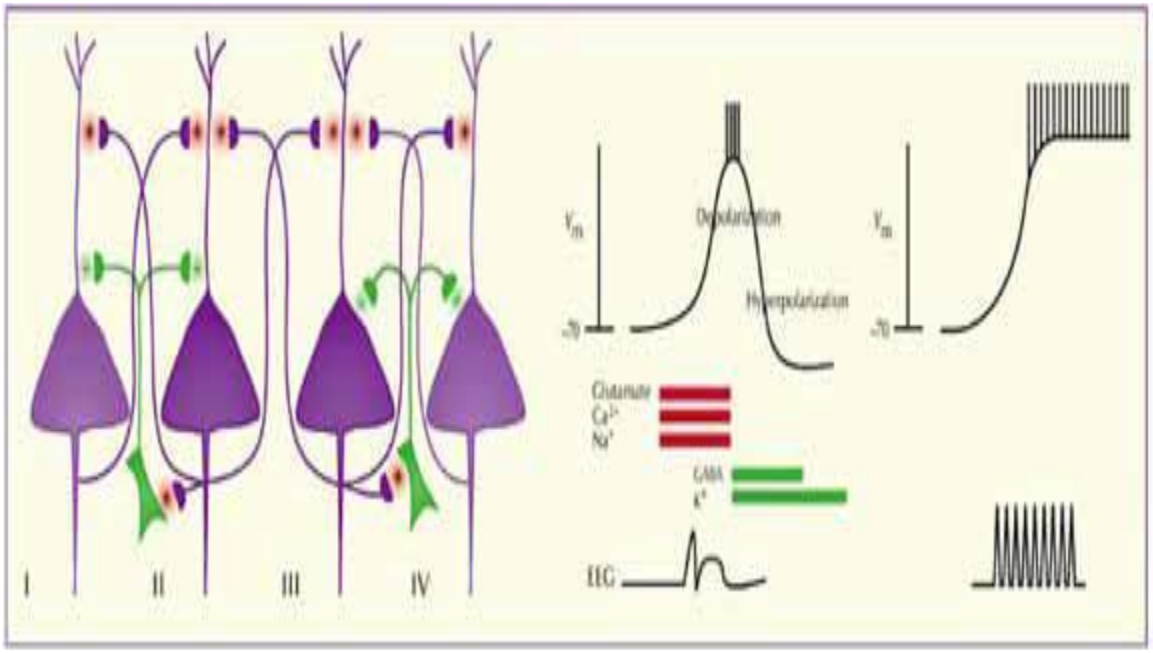


Figure 3: Paroxysmal depolarization shift (PDS)[31]

[When a PDS occurs as an abnormally prolonged run of action potentials during sustained membrane depolarization in a single neuron, as shown in the upper trace in **B**, the event is detectable only with microelectrodes; increased glutamate concentration is associated with influx of cations initially, followed by increased GABA concentration with efflux of potassium. When PDSs in a large number of neurons are synchronized for less than 200 ms, as shown in **A**, these electrical potentials may summate as a spike-wave complex that is recorded with macroelectrodes, as shown in the lower trace in **B**. When sustained repetitive firing of PDSs in a large number of neurons becomes synchronized for many seconds or longer, an electrographic seizure occurs, as shown in **C**.]

Repetitive neuronal firing probably underlies the interictal and ictal unit and local field recording of high frequency oscillations.[32] The tendency of individual neurons to

enter pathological states in which PDSs are generated can be based on intrinsic neuronal properties, such as dysfunctional ionophores in the genetically determined channelopathies (**Figure 4**),[33] or on extrinsic mechanisms such as inadequate inhibitory neurotransmitter concentrations or exposure to excessive concentrations of excitatory amino acids or exogenous excitotoxins. However, large groups of neurons must generate PDSs simultaneously to account for the episodic nature of seizures.

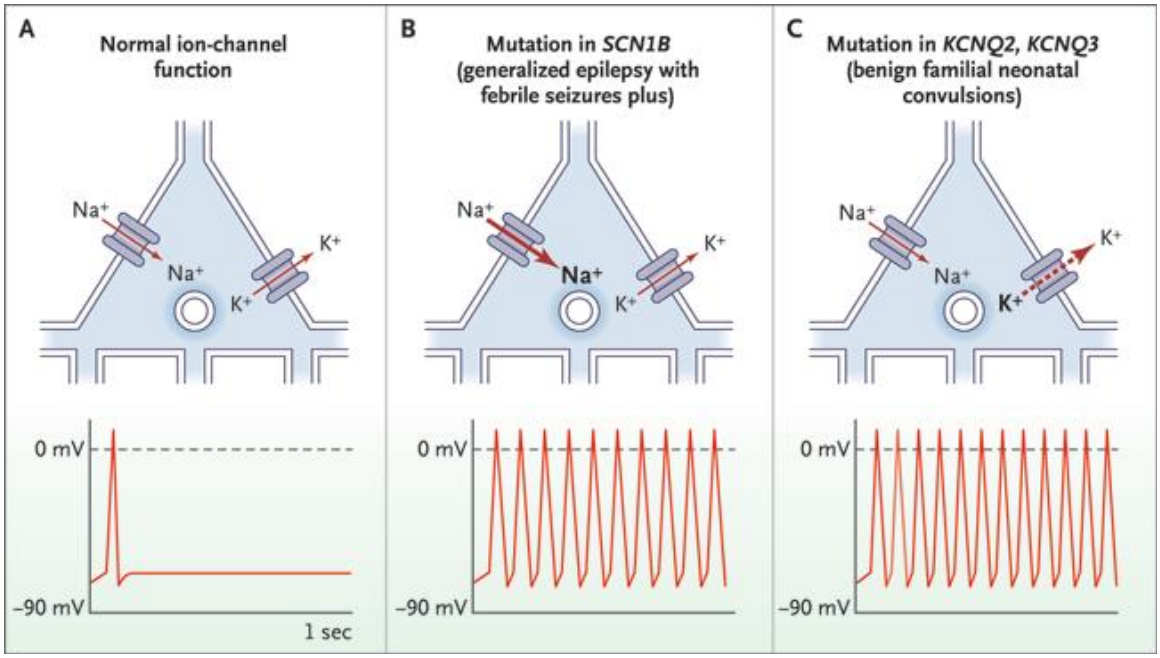


Figure 4: Genetic channelopathies in epilepsy[33]

[(**A**) Genetic channelopathies can support the occurrence of paroxysmal depolarization shifts (PDS) by altering the usual balance of Na+ and K+ ion conductance across neuronal membranes. Increased Na+ conductance (**B**, upper panel) creates a situation in which a single action potential initiates sustained depolarization as a PDS (**B**, lower panel). Decreased K+ conductance (**C**, upper panel) also can predispose to PDS.]

In experimental models of generalized epilepsies, this widespread epileptic synchronization of interictal and ictal PDSs is based on intrathalamic synchronization that drives thalamocortical relay neurons to synchronize the bihemispheric cortical neuronal discharges (**Figure 5**).[33] Under normal conditions, brainstem monoaminergic

projections synchronize the thalamic reticular neurons into cycles of slow waves, and GABAergic projections of these neurons synchronize thalamocortical relay neurons into cycles of slow waves, resulting in synchronized glutamatergic thalamocortical projections to widespread cortical areas that generate the electroencephalography (EEG)-recorded slow waves of non-REM sleep and also sleep spindles.

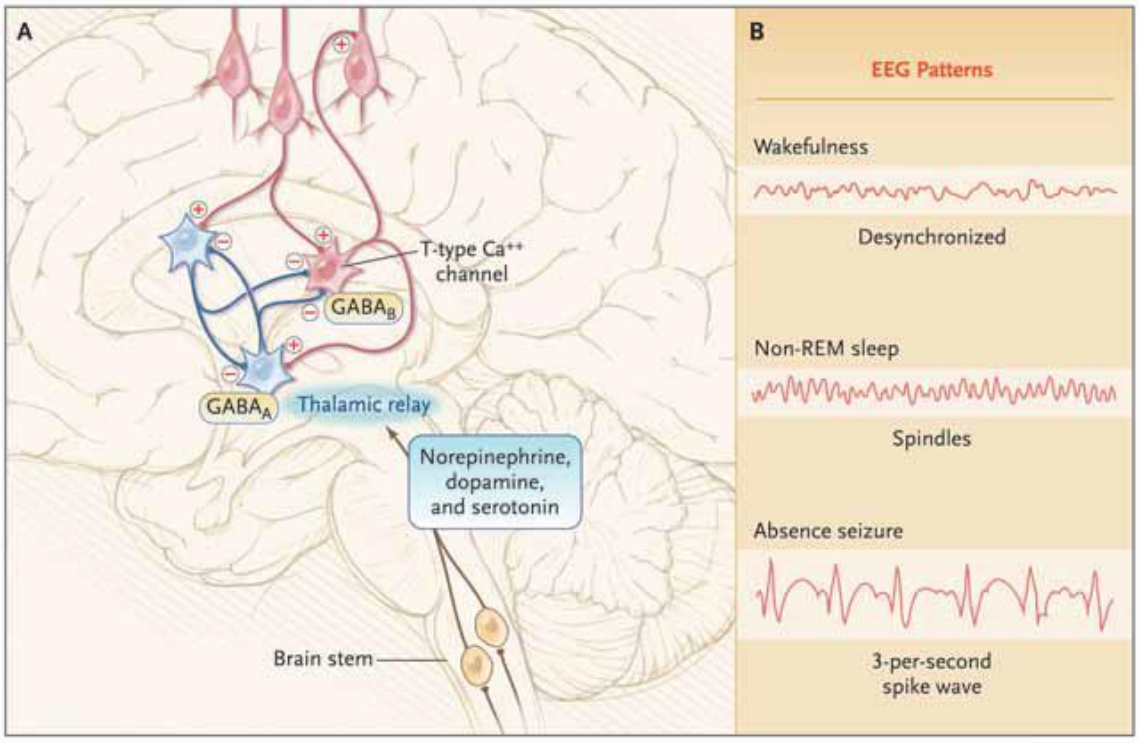


Figure 5: Thalamocortical circuits in generalized epilepsies[33]

[In generalized epilepsies, repetitive paroxysmal depolarization shifts (PDSs) in thalamic reticular neurons synchronize PDSs in thalamocortical relay neurons, which in turn synchronize PDSs in cortical neurons, thus generating EEG-recorded spike-wave discharges and sometimes absence seizures. Presumably, a structurally normal complement of thalamic and cortical neurons and their pathways are able to generate spike-wave discharges and absence seizures due to genetically based dysfunction of channels, receptors, or other neurochemical elements.]

In experimental models of partial epilepsies, intracortical mechanisms of synchronization operate during ictal discharges (**Figure 6**).[33] There is evidence that in

certain epilepsies specific channelopathies operate to initiate PDSs in individual neurons and produce seizures through normal mechanisms of interneuronal synchronization, while other epilepsies appear to require abnormal interneuronal pathways to generate pathological synchronization. Epileptic reorganization of the hippocampal formation can be caused by a variety of insults that cause loss of vulnerable neurons in experimental models, but etiologic insults only occasionally are evident in human temporal lobe epilepsy (TLE).

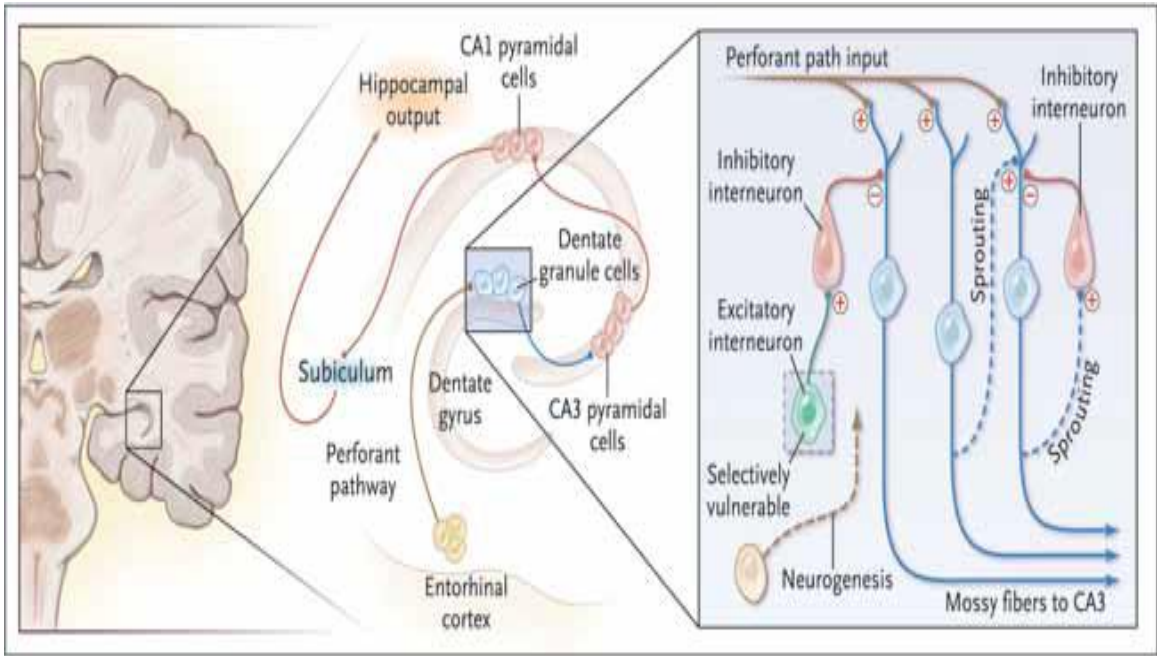


Figure 6: Epilepsy pathophysiology in mesial temporal lobe epilepsy (TLE) with hippocampal sclerosis[33]

[This epileptic reorganization includes sprouting of mossy-fiber axons of dentate granule cells; dentate granule cells project to CA3 neurons, and increased activity in mossy-fiber projections can support excessive excitation of CA3 neurons. Thus, according to this hypothesis, normal excitatory inputs from the entorhinal cortical neurons to these reorganized dentate granule cells induce excessive excitation of CA3 neurons in the setting of inadequate inhibition, to initiate sustained repetitive firing in downstream CA1 neurons as a focal seizure with the potential to propagate more widely. Several experimental models of TLE generate the same histopathological findings in the animal hippocampus that are observed in the human hippocampal sclerosis.]

Classification

1981 Classification[33]

There are many highly individual aspects of the seizures, interictal dysfunctions, and associated cerebral and systemic conditions experienced by persons with epilepsy. Nonetheless, it is obvious that some of these characteristics cluster across individuals as particular types of epilepsy. Ideally, every person with epilepsy could be assigned to one type of epilepsy, and the various types of epilepsy could be classified into a neurobiologically based, clinically relevant organizational schema of the epilepsies. This ideal taxonomy of the epilepsies has yet to be devised, and clinical epileptologists must use the current epilepsy classifications to guide diagnostics and therapeutics. At this time, the most widely accepted classification of epilepsy types is that published by the ILAE in 1981 (**Table 1**).

Table 1: ILAE 1981 Classification of Epilepsies[33]

Type	Epilepsies	Clinical Specifications
1	**Localization-related (focal, local, partial) epilepsies and syndromes**	1.1 Idiopathic (with age-related onset) • Benign childhood epilepsy with centrotemporal spikes • Childhood epilepsy with occipital paroxysms • Primary reading epilepsy 1.2 Symptomatic • Chronic progressive epilepsia partialis continua of childhood (Kojewnikow syndrome) • Syndromes characterized by seizures with specific modes of precipitation • Temporal lobe epilepsies (amygdalo-hippocampal, lateral temporal) • Frontal lobe epilepsies (supplementary motor, cingulate, anterior frontopolar, orbitofrontal, dorsolateral, opercular, motor cortex) • Parietal lobe epilepsies

		• Occipital lobe epilepsies 1.3 Cryptogenic • Same syndromes as for symptomatic localization-related epilepsies, but without known etiology)
2	**Generalized epilepsies and syndromes**	2.1 Idiopathic (with age-related onset) • Benign neonatal familial convulsions • Benign neonatal convulsions • Benign myoclonic epilepsy in infancy • Childhood absence epilepsy (pyknolepsy) • Juvenile absence epilepsy • Juvenile myoclonic epilepsy • Epilepsy with grand mal (GTCS) seizures on awakening • Other generalized idiopathic epilepsies not defined above • Epilepsies with seizures precipitated by specific modes of activation 2.2 Cryptogenic or symptomatic • West syndrome • Lennox-Gastaut syndrome • Epilepsy with myoclonic-astatic seizures • Epilepsy with myoclonic absences 2.3 Symptomatic 2.3.1 *Nonspecific etiology* • Early myoclonic encephalopathy • Early infantile epileptic encephalopathy suppression burst • Symptomatic generalized epilepsies not defined above 2.3.2 *Specific syndromes* • Epileptic seizures that complicate a specific disease state
3	**Epilepsies and syndromes undetermined whether focal or generalized**	3.1 With both generalized and focal seizures • Neonatal seizures • Severe myoclonic epilepsy in infancy • Epilepsy with continuous spike-waves, slow wave sleep • Acquired epileptic aphasia (Landau-Kleffner syndrome) • Other undetermined epilepsies not defined above 3.2 Without unequivocal generalized or focal features. • All cases with generalized tonic-clonic seizures in which clinical and EEG findings do not permit classification as clearly generalized or localization related, such as in many cases of sleep grand mal (GTCS) are considered not to have unequivocal generalized or focal features.

4	**Special syndromes**	4.1 Situation-related seizures (Gelegenheitsanfälle) ○ Febrile convulsions ○ Isolated seizures or isolated status epilepticus ○ Seizures occurring only when there is an acute metabolic or toxic event due to factors such as alcohol, drugs, eclampsia, nonketotic hyperglycemia

In addition to defining accepted epilepsy types, this taxonomy used 2 dichotomous principles to organize the epilepsy types. The partial (or focal, or localization-related) versus generalized dichotomy was determined by whether the individual had partial-onset or generalized- onset seizures, respectively. The idiopathic (or primary) versus symptomatic (or secondary, or acquired) dichotomy was determined by whether the individual had seizure intrinsic to his or her brain structure-function in the absence of any brain insult, or had a brain insult that precipitated epileptogenesis, respectively. A special category of the symptomatic group was persons with seizures of cryptogenic etiology who had indirect evidence of having had a causative brain insult, although the precise insult could not be determined. Characteristically, the idiopathic epilepsies had no interictal evidence of brain injury, so interictal intelligence and other functions typically are normal or nearly normal. Symptomatic partial epilepsies often feature interictal dysfunctions localized to the injured area, which includes the ictal onset zone, with mental retardation or developmental delay in many of those with symptomatic generalized epilepsies (eg, delayed verbal recall deficits in those with left-sided mesial temporal lobe epilepsy-hippocampal sclerosis, a symptomatic and usually cryptogenic partial epilepsy).

2010 Classification[34]

Further, more efforts to improve epilepsy classification were done in 2010 as summarized in **Table 2**.

Table 2: ILAE 2010 Classification of Epilepsies[34]

Organizational Group	Electroclinical Syndrome
Neonatal onset group	○ Benign familial neonatal epilepsy (BFNE) ○ Early myoclonic encephalopathy (EME) ○ Early myoclonic encephalopathy (EME)
Infancy onset group	○ Epilepsy of infancy with migrating focal seizures ○ West syndrome ○ Myoclonic epilepsy in infancy (MEI) ○ Benign infantile epilepsy ○ Benign familial infantile epilepsy ○ Dravet syndrome ○ Myoclonic encephalopathy in nonprogressive disorders
Childhood onset group	○ Febrile seizures plus (FS+) (can start in infancy) ○ Panayiotopoulos syndrome ○ Epilepsy with myoclonic atonic (astatic) seizures ○ Benign epilepsy with centrotemporal spikes (BECTS) ○ Autosomal-dominant nocturnal frontal lobe epilepsy (ADNFLE) ○ Late onset childhood occipital epilepsy (Gastaut type) ○ Epilepsy with myoclonic absences ○ Lennox-Gastaut syndrome ○ Epileptic encephalopathy with continuous spike-and-wave during sleep (CSWS) ○ Landau-Kleffner syndrome (LKS) ○ Childhood absence epilepsy (CAE)
Adolescence – Adult	○ Juvenile absence epilepsy (JAE) ○ Juvenile myoclonic epilepsy (JME) ○ Epilepsy with generalized tonic–clonic seizures alone ○ Progressive myoclonus epilepsies (PME) ○ Autosomal dominant epilepsy with auditory features (ADEAF) ○ Other familial temporal lobe epilepsies
Less specific age relationship	○ Familial focal epilepsy with variable foci (child to adult) ○ Reflex epilepsies
Distinctive constellations	○ Mesial temporal lobe epilepsy with hippocampal sclerosis (MTLE with HS)

	○ Rasmussen syndrome ○ Gelastic seizures with hypothalamic hamartoma ○ Hemiconvulsion–hemiplegia–epilepsy
Epilepsies that do not fit into any of these diagnostic categories	
Epilepsies attributed to and organized by structural-metabolic causes	
Epilepsies of unknown cause	
Conditions with epileptic seizures that are traditionally not diagnosed as a form of epilepsy per se	○ Benign neonatal seizures (BNS) ○ Febrile seizures (FS)

The partial-generalized and idiopathic-cryptogenic dichotomies were discarded, and the epilepsy types were organized by typical developmental stage at seizure onset (eg, neonatal, childhood) and etiology. The established types of epilepsy are mainly the same as those recognized by the 1989 classification, although more developed and specific syndromes of neonatal seizure onset are included in the 2010 classification. The new epilepsy classification has been described as multifactorial and flexible, with emphases on age at onset and etiology, and also as a work-in-progress and incomplete. Epileptologists will need to track this transitional period in epilepsy classification systems, but fortunately the core knowledge of classification continues to be in recognition of the individual epilepsy types.

2016 Classification[35]

The International League Against Epilepsy (ILAE) presented a revised operational classification of seizure types (**Figure 7**). The purpose of such a revision is to recognize that some seizure types can have either a focal or generalized onset, to allow classification when the onset is unobserved, to include some missing seizure types and to

adopt more transparent names. Because current knowledge is insufficient to form a scientifically-based classification, the 2016 classification is operational (practical) and based upon the 1981 Classification, extended in 2010. The new classification does not represent a fundamental change, but allows greater flexibility and transparency in naming seizure types. Changes include:

1. “partial” becomes “focal”;

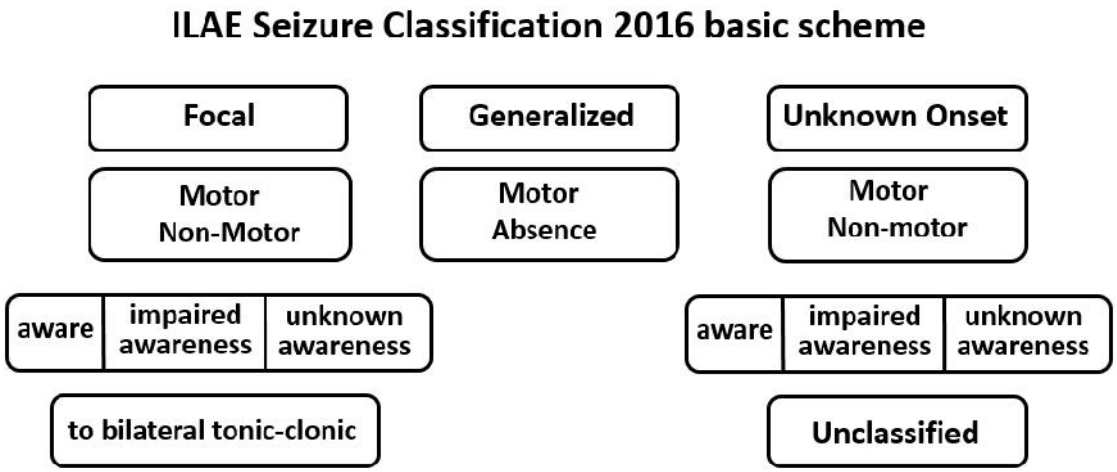
2. Seizures of unknown onset can still be classified;

3. Awareness is used as a classifier of focal seizures;

4. The terms dyscognitive, simple partial, complex partial, psychic, secondarily generalized are eliminated;

5. Focal tonic, clonic, atonic, myoclonic and epileptic spasms seizure types are recognized, along with bilateral versions of these seizure types.

6. Addition of new generalized seizure types: absence with eyelid myoclonia, myoclonic absence, myoclonic-atonic, clonic-tonicclonic, epileptic spasms. Epileptic spasms can thus be focal, generalized or unknown. 7. Bilateral tonic-clonic seizure replaces secondarily generalized seizure.



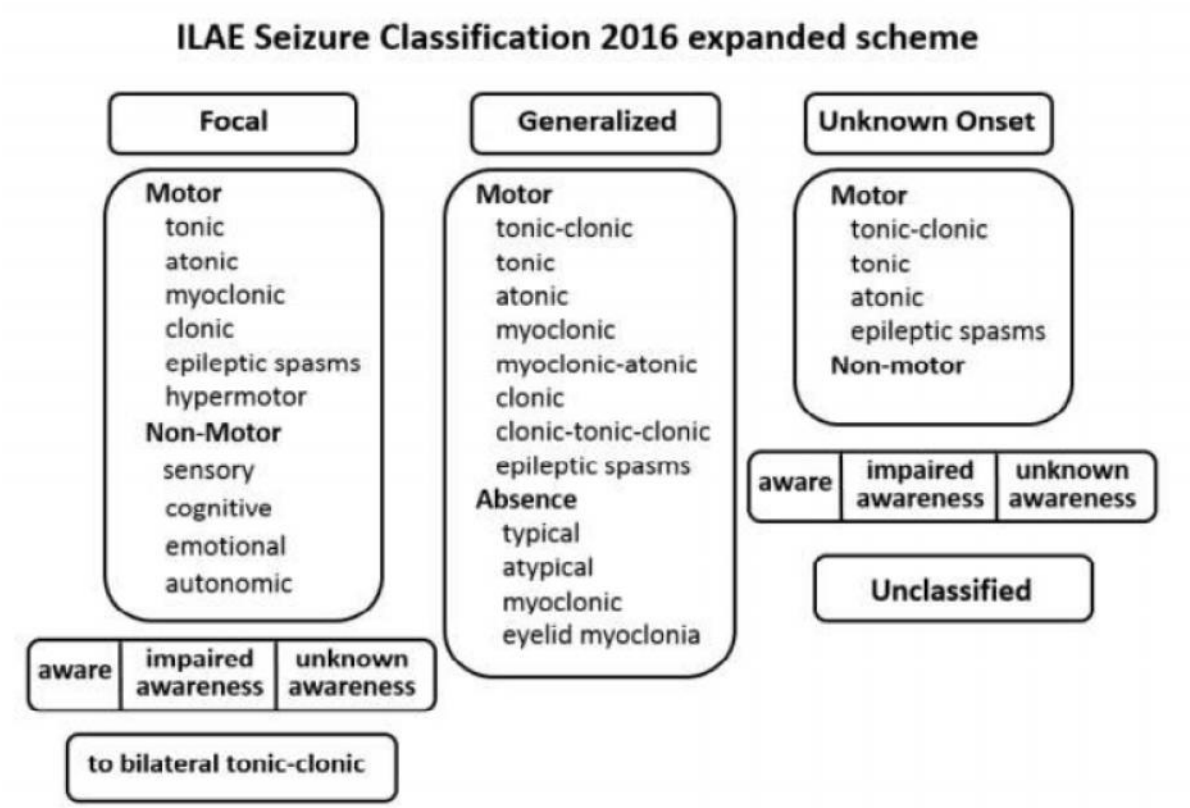


Figure 7: The ILAE 2016 Operational Classification of Seizure Types: Basic and Expanded Scheme[35]

The classification attempts first to determine whether the initial manifestations of the seizure are focal or generalized. The onset may be missed or obscured, in which case the seizure is of unknown onset. Subtypes are described in the glossary of terms below. The classification of an individual seizure can stop at any level. It may be designated a "focal" (or "generalized") seizure, with no other elaboration, or a "focal sensory seizure," "focal motor seizure," "focal tonic seizure," or any other listed combination. Additional classifiers are encouraged, but optional, and their use may depend upon the experience and purposes of the person classifying the seizure.

Focal seizures are subdivided as those with motor and non-motor signs and symptoms. If both motor and non-motor signs are present at the seizure start, the motor

signs will usually dominate, unless non-motor (e.g., sensory) symptoms and signs are very prominent. Focal seizures can be associated with a variety of symptoms, signs and behaviors, a key one of which is impairment of awareness, responsiveness, recall or consciousness. Impaired awareness is employed as shorthand for presence of any of these features. A “focal aware seizure” corresponds to the 1981 designation “simple partial seizure,” and it implies that awareness, recall, responsiveness and consciousness are all intact. Any significant impairment of these clinical states causes a focal seizure to be classified as having impaired awareness. It may be convenient to refer to this seizure type as “focal unaware,” but in doing so it is important to recognize that awareness may be impaired, rather than absent. A “focal seizure with impaired awareness” corresponds to the 1981 designation “complex partial seizure.” If the state of awareness is unknown, then the focal seizure is classified as being “with unknown awareness.” When a focal seizure presents with more than one of the classifiers under the focal heading, then the presumption is that the one presenting early and prominently will have primacy, since it reflects the most important involved regions or networks of brain. If more than one sequential feature is prominent, then it is best to consider a propagation pattern within one seizure type or among multiple sequentially developing seizure types. The exception is characterization of awareness, which can be added to any focal seizure. It is acceptable to omit the implied term “non-motor” for focal sensory, cognitive, emotional or autonomic seizures. The term “aware” may also be omitted for seizure types such as sensory for which awareness is implied. Order of terms is not crucial, such that “focal aware tonic seizure” means the same as does “focal tonic seizure with preserved

awareness." Where a word can be unambiguously assumed, it may be omitted, for example, "generalized tonic seizure," rather than "generalized motor tonic seizure."

The seizure type "focal to bilateral tonic-clonic" is a special seizure type, corresponding to the prior phrase "partial onset with secondary generalization." Focal to bilateral tonic-clonic reflects a propagation pattern of a seizure, rather than a unitary seizure type, but it is such a common and important presentation that the separate categorization was continued. Other focal seizure types, such as tonic or myoclonic may progress (propagate) to bilateral tonic-clonic seizures.

Generalized seizures are divided into motor and absence seizures. Further subdivisions are similar to those of the 1981 classification, with addition of myoclonic-atonic seizures, common in epilepsy with myoclonic-atonic epilepsy (Doose syndrome), clonic-tonic-clonic seizures common in juvenile myoclonic epilepsy, myoclonic absence and absence with eyelid myoclonia seizures seen in the syndrome described by Jeavons and elsewhere.

"Unknown onset" seizures are not truly separate types of seizures, but rather placeholders for seizure types for which the onset is unknown. A wife may awaken to her husband's first tonic-clonic seizure, which would be classified as an onset unknown tonic-clonic seizure. If a subsequent seizure is observed with a clear focal onset, then the seizure type would be reclassified as a "focal to bilateral tonic-clonic seizure."

An "unclassified seizure" connotes a seizure with no specification as to nature of onset, presence of motor or non-motor features and no details about degree of awareness. If any of these features are known, then those details should be employed to partially

classify the seizure. Not every seizure will fit into this classification, so a choice for "other" can be inferred in each category.

Clinical Diagnosis

The first seizure that brings a patient to the attention of a physician is usually a tonic–clonic seizure. If there is no suggestion of a provoked seizure or an acute cause, including substance abuse, lack of sleep, and medical disease, early-stage epilepsy is likely. Particularly in those who seem to have had several unprovoked tonic-clonic seizures, a careful history will often bring to light additional seizures such as absence, myoclonic, and, more often, complex partial seizures, which not only confirm the diagnosis of epilepsy but often allow diagnosis of the epilepsy syndrome.

The diagnosis of a seizure can be made clinically in most cases by obtaining a detailed history and performing a general clinical examination with emphasis on neurological and psychiatric status. An eyewitness account of a typical seizure(s), age and external circumstances at onset, frequency of each type of seizure, and longest and shortest intervals between them should be recorded.

A seizure diary helps to ascertain treatment response. The history should cover the existence of prenatal and perinatal events, spontaneous abortions, seizures in the newborn period, febrile seizures, any unprovoked seizures, and epilepsies in the family (**Table 3**). The existence of an aura should be ascertained, and conditions that may have precipitated the seizure in the opinion of the patient should be documented. A history of prior head trauma, infection, or toxic episodes must be sought and evaluated. A family history of seizures or neurological disorders is significant.

Table 3: History taking in a patient with new-onset epilepsy

Patient	Seizure onset, course, time of day. Earlier seizures? Postictal paresis, aphasia, speech problem, tongue bite, tiredness?
Observer	Onset, course, duration, eyes open or closed, facial color, facial and upper chest petechiae. Postictal speech, paresis, confusion and its duration, behavior?
Physician	Caution: Frequent early diagnostic errors include nonepileptic psychogenic seizures, convulsive syncope with myoclonus, and REM behavioral disorder, the latter mostly in elderly men. If in doubt that it is epilepsy, better wait for confirmation through EEG or by observing another seizure. A delayed diagnosis usually causes no problem, while a premature and erroneous diagnosis of epilepsy may have grave implications.

Once the seizures are classified (e.g., simple, complex partial, partial-onset GTC or generalized absence, myoclonic seizure or generalized-onset GTC) and, if possible, the epilepsy (idiopathic or symptomatic) or the epilepsy syndrome (e.g., temporal lobe epilepsy, generalized juvenile myoclonic epilepsy) is classified, appropriate studies are ordered including an electroencephalogram (EEG)[36] (**Figure 8**), magnetic resonance imaging (MRI)[37] (**Figure 9** and **Table 4**), and serum glucose, sodium, magnesium, and calcium levels (**Table 5**).

The EEG between seizures (interictal) in primarily generalized tonic-clonic seizures is characterized by symmetric bursts of sharp and slow, 4 to 7 Hz activity. Focal epileptiform discharges occur in secondarily generalized seizures. In absence seizures, spikes and slow waves appear at a rate of 3/second. Interictal temporal lobe foci (spikes or slow waves) occur with complex partial seizures of temporal lobe origin. Because an

EEG taken during a seizure-free interval is normal in 30% of patients, one normal EEG does not exclude epilepsy.

A second EEG performed during sleep in sleep-deprived patients reveals epileptiform abnormalities in half of patients whose first EEG was normal. Rarely, repeated EEGs are normal, and epilepsy may have to be diagnosed on clinical grounds. When the seizures are focal or an EEG is focally abnormal, when seizures begin in adulthood, or the physical examination reveals focal pathological symptoms or signs, MRI is indicated to detect structural lesions caused by, for example, cortical malformation, traumatic brain injury, brain tumor, and cerebrovascular disease, which are the most common causes of symptomatic epilepsy.

In our view, MRI is also useful in generalized epilepsy or generalized seizures to search for dual pathology, for example, an unsuspected brain lesion.[37] A lumbar puncture should be performed if fever and stiff neck accompanying newonset seizures suggest meningitis, subarachnoid hemorrhage, or encephalitis.

Recommendation:

- Every patient with newly diagnosed epilepsy should undergo MRI to identify structural causes of epilepsy
- EEG to assist in the diagnosis of the syndrome.
- If the first EEG is normal, order an EEG during sleep
- If the first MRI scan is normal, repeat in the case of drug-resistant epilepsy as a first step to explore surgical options.

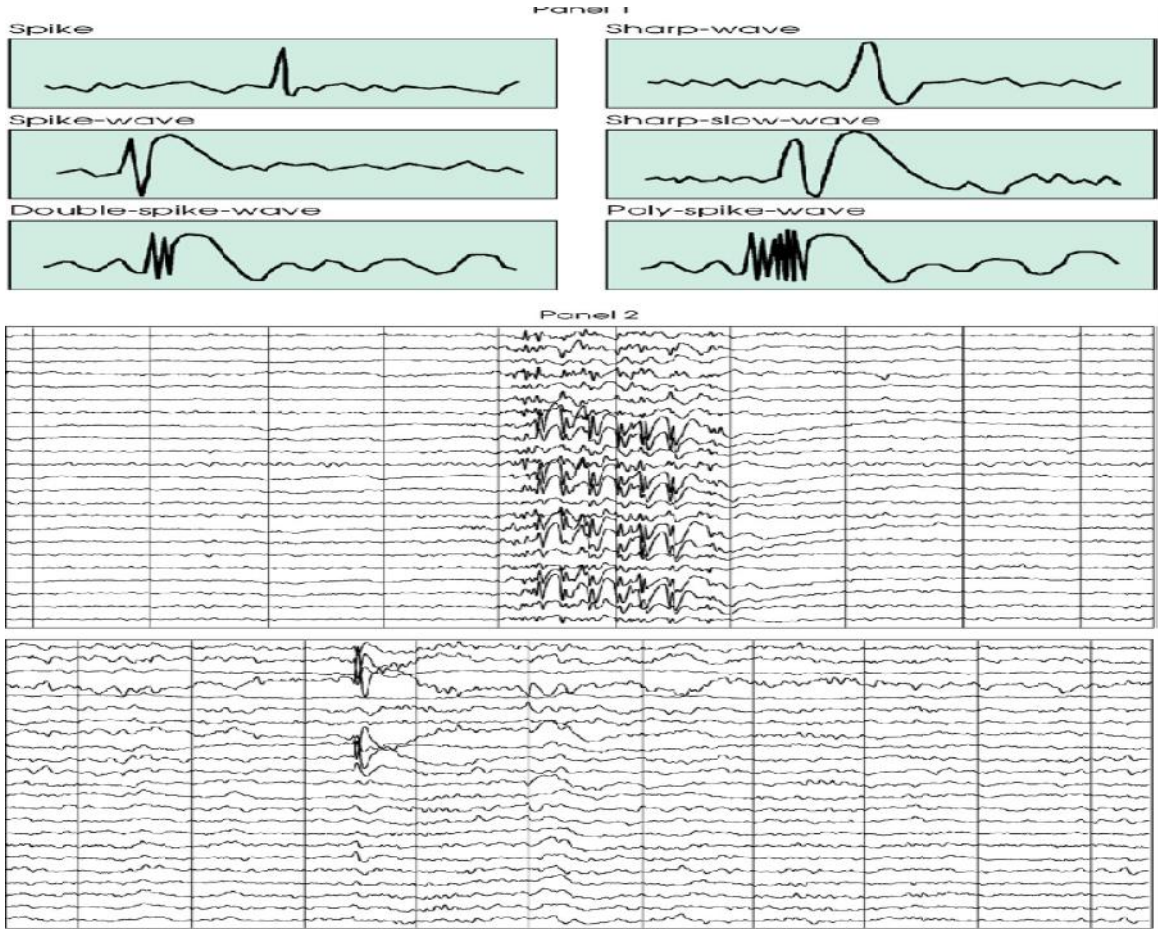


Figure 8: Electroencephalography[36]

Panel 1: Interictal discharges are distinctive waves or complexes that can be recorded between seizures in the EEGs of individuals with epilepsy. Generally brief in duration, they may have a variety of morphologies described as sharp wave, spike wave, or spike-and-slow wave.

Panel 2, top: A generalized discharge of bilateral spike-and-wave activity, predominantly in the frontal region.

Panel 2, bottom: Single sharp-slowwave, right mesial temporal region.]

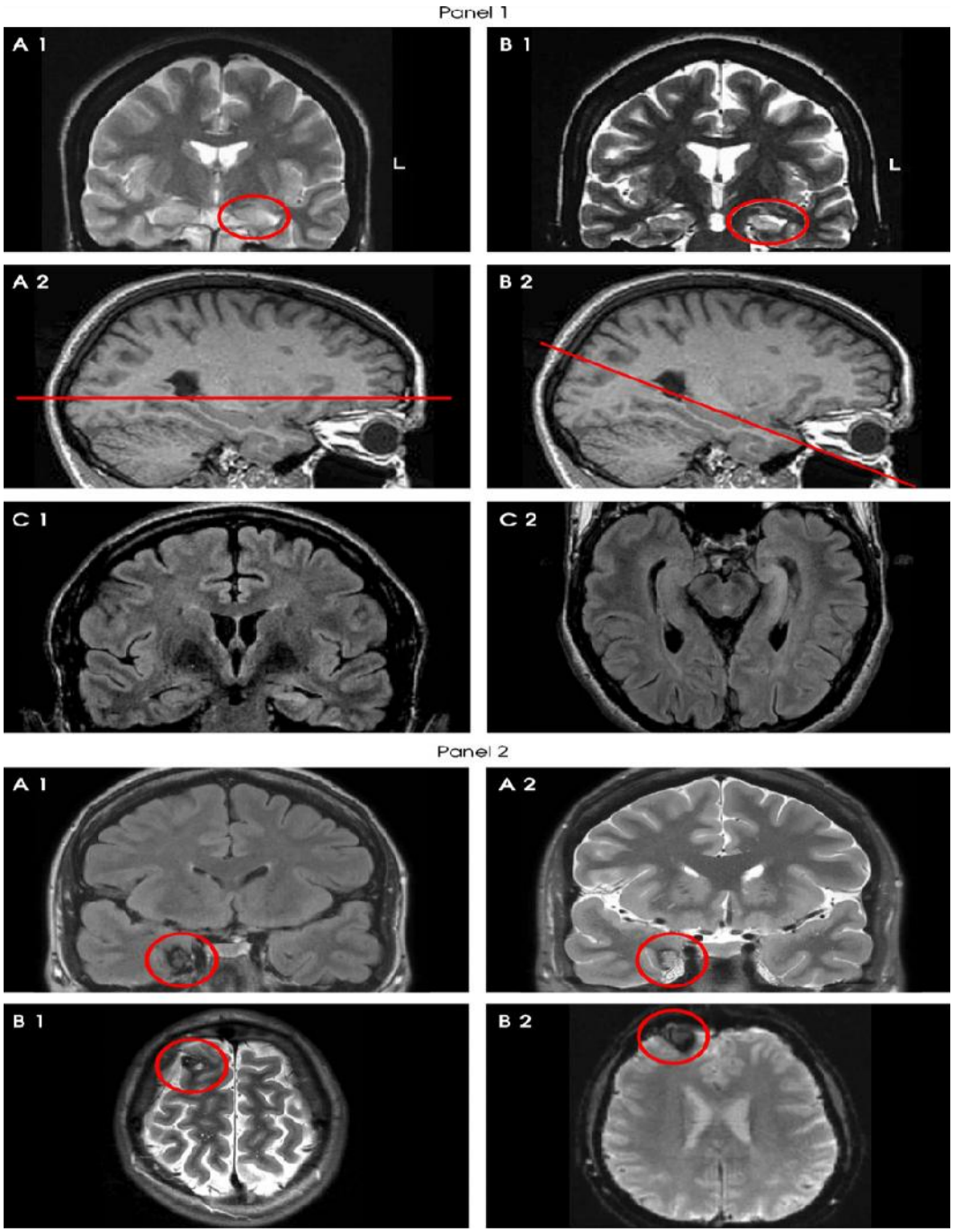


Figure 9: Magnetic resonance imaging (MRI) in epilepsy[37]

[*Panel 1*: A1, Ammonshornsclerosis left, standard coronal angulation; A2, standard coronal angulation; B1, Ammonshornsclerosis (syn.: mesial temporal sclerosis) left, coronal temporal angulation; B2, coronal temporal angulation; C1, flair sequence coronal and axial, temporal angulation. *Panel 2*: Cavernoma; A1, mesio-temporal right, flair sequence; A2, T2 weighted; B1, frontal right, T2 weighted; B2, T2* weighted (pronounced effect of old blood).]

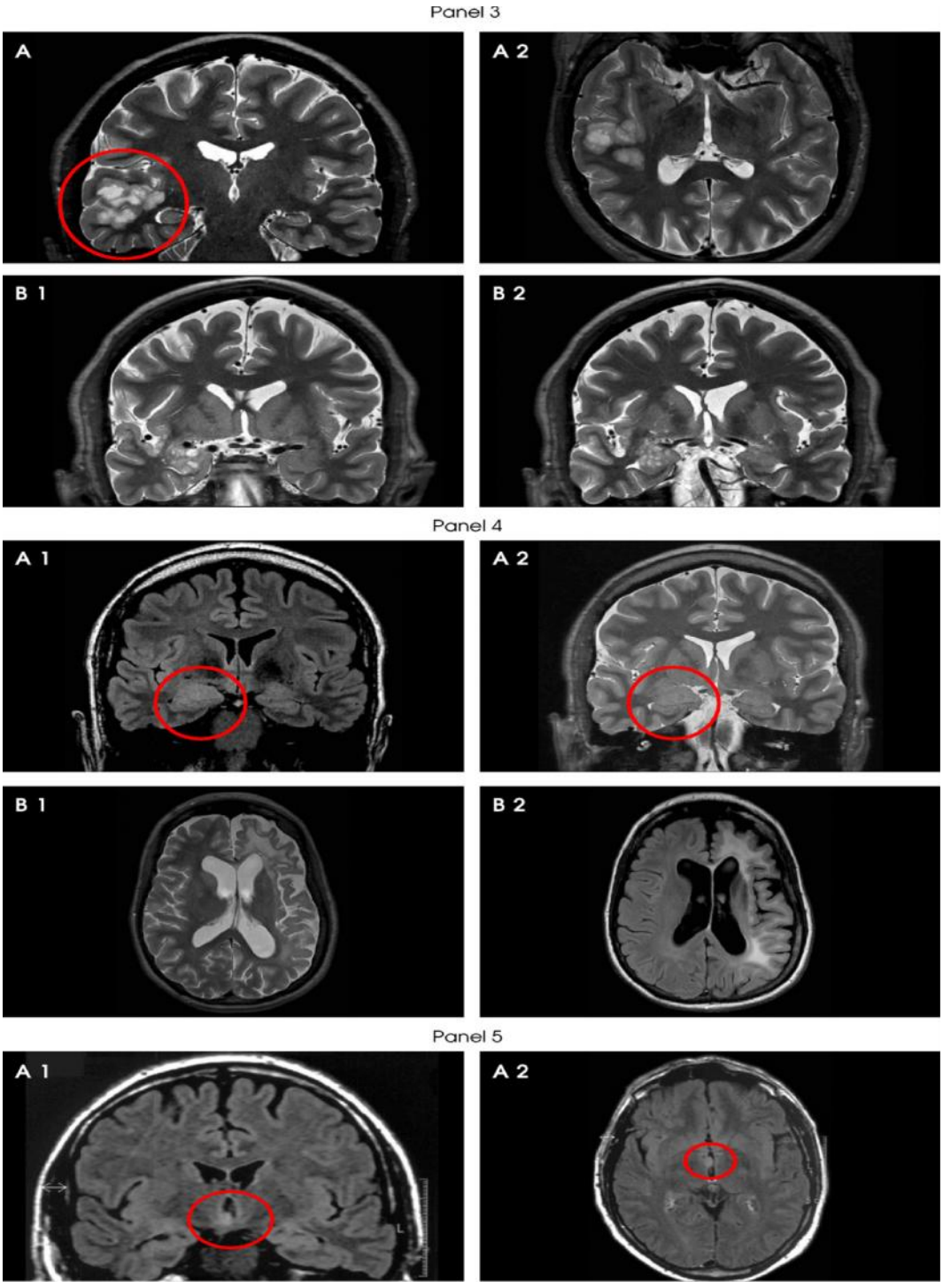


Figure 9: continued

[*Panel 3*: Developmental tumors A, dysembryoplastic neuroepithelial tumor; temporo-lateral right, B1,2, ganglioglioma temporo-mesial right. *Panel 4*: Chronic inflammation: A 1,2 = nc. amygdala right; B 1,2, Rasmussen's Encephalitis, left hemisphere. Panel 5: A1,2, Hypothalamic hamartoma.]

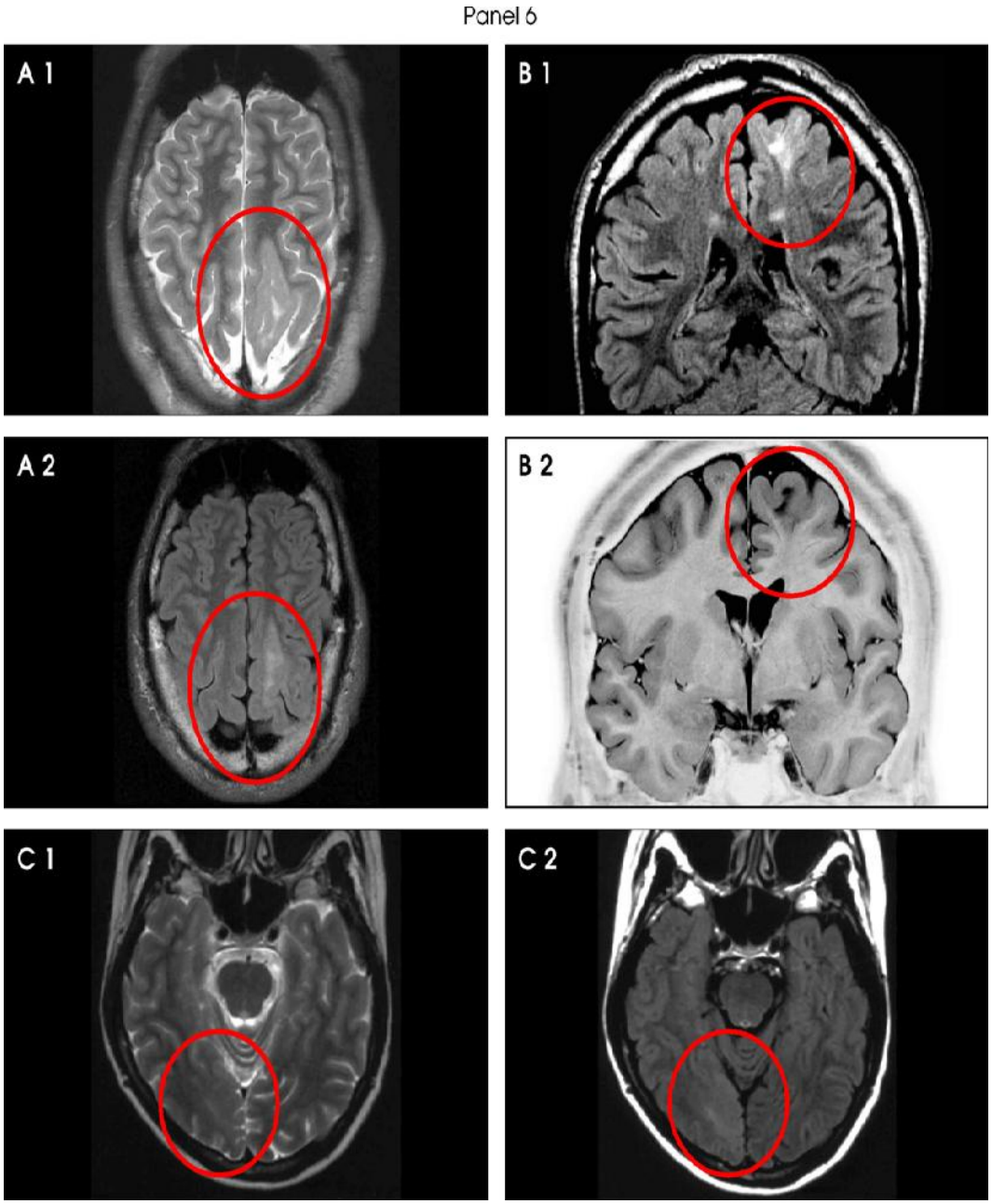


Figure 9: continued

[*Panel 6*: Cortical developmental abnormality left parietal (A+B) and occipital C (cortical dysplasia Type IIb (Palmini): A1, T2 weighted axial; B2, flair sequence coronal; A2, flair sequence coronal; B1, flair sequence coronal; B2, inversion recovery sequence coronal. C1, T2 weighted; C2, flair sequence (C: courtesy of Department of Radiology, University Bonn, Germany).]

Table 4: Suggested MRI protocols for epilepsy patients

Temporal lobe epilepsy	1. Hippocampal oriented T2-weighted (coronal + axial) 2. Hippocampal oriented fluid-attenuated inversion recovery (FLAIR) (coronal + axial) 3. Isotropic T1-weighted three-dimensional sequence (MPRage) 4. (Gadolinium contrast-enhanced T1-weighted image if non-contrast-enhanced image is inconclusive) 5. T2*-weighted sequence
Extratemporal lobe epilepsy	1. AC–PC oriented T2-weighted (coronal + axial) 2. AC–PC oriented FLAIR (coronal + axial) 3. Isotropic T1-weighted three-dimensional sequence (MPRage) 4. (Gadolinium contrast-enhanced T1-weighted image if non-contrast-enhanced image is inconclusive) 5. T2*-weighted sequence
Special protocols: Rationale	1. T2 relaxometry hippocampal signal abnormalities 2. Magnetic resonance spectroscopy detection of metabolic abnormalities 3. Diffusion tensor imaging (DTI) investigation of fiber tracts 4. Functional MRI investigation of eloquent cortical areas 5. Three-dimensional sequences automated voxel-based analyses 6. Magnetic resonance angiography investigation of brain vascularisation

Table 5: Studies in patient with new-onset epilepsy

MRI	Each patient with new-onset epilepsy should have an MRI (temporal angulation, T1,T2, FLAIR, coronal and axial) to detect structural lesions caused by for example cortical malformation, traumatic brain injury, brain tumor, and cerebrovascular disease, which are the most common causes of symptomatic epilepsy. Contrast media, inversion recovery, fast field echo and 3D only in special cases. Even in idiopathic epilepsy, MRI is recommended to diagnose unsuspected dual pathology.
EEG	Each patient with newonset epilepsy should have an EEG. EEG is most valuable within 24 h of the seizure. Information gain is optimal up to the 4th EEG, if no paroxysmal interictal discharges are found, repeat EEG during sleep. 24-hour EEG most meaningful in a patient with frequent seizures who can be expected to have seizures during the 24 h recording. Interictal EEG discharges may support diagnosis of epilepsy syndrome.
Head X-ray	Obsolete
Clinical Chemistry	Routine work-up, creatinkinase, Vitamin B6 if seizures are unresponsive to AEDs, even in adults. CSF, only when infectious disorders are suspected. Creatinkinase increased within 12-24 h, prolactin increased within 30 min.

B. ANTIEPILEPTIC DRUGS (AEDs)

First, Second and Third generation AEDs

There are approximately 22 AEDs (antiepileptic drugs) currently available in the United States. Prior to 1993, the primary AEDs were phenobarbital, phenytoin, carbamazepine, and valproic acid. Since 1993, numerous medications have become available, allowing providers to better customize pharmacotherapy to the individual patient. The newer AEDs are referred to as second- and third generation AEDs.

Antiepileptic drugs are the simplest and the safest means of controlling epilepsy. Various first-generation antiepileptic drugs-phenytoin, carbamazepine, valproic acid, phenobarbital, clonazepam, primidone, and ethosuximide are used widely in Asian countries and monotherapy is common.[38] The particular drug used depends on the medical culture and practices in each country. Second-generation antiepileptic drugs, such as lamotrigine, gabapentin, tiagabine, felbamate, vigabatrin, or topiramate, are used widely in Malaysia, China, and Singapore and in some of the economically less developed countries, including the Philippines and Vietnam.[39] Clobazam, oxcarbazepine, and levetiracetam are available in large Asian countries, such as India, but are prohibitively expensive. Access to drugs is probably easier in Asia than in Africa but this varies widely according to the context (degree of development, urbanisation, etc). Although first-generation antiepileptic drugs are predominant, there are availability and accessibility problems in several places. The average annual cost (direct and indirect) of outpatient treatment of epilepsy is US$47 per patient. In general, the cost of phenobarbital in southeast Asia is 2·7 times higher than in Europe,[40] and two to six times higher than in sub-Saharan Africa. The annual cost incurred in emergency and inpatient

management in India is estimated to be US$810·50 and US$168·30, respectively, for all the patients attending a secondary hospital.[41] In general, the cost of treatment is much lower than productivity losses, and it would be cost efficient for governments or societies to invest in epilepsy treatment.[41]

Mechanism of Action

For most AEDs, the mechanisms by which they exert an anticonvulsant effect are not entirely understood. The primary mechanisms of action for these drugs involve decreasing the excitation of neurons by blocking Na^+ and/or Ca^{2+} channels or antagonizing glutamate receptors. Some medications increase the inhibition of neurons by increasing or enhancing γ-aminobutyric acid (GABA).[41] The mechanisms of action of the currently available AEDs are summarized in **Table 6**.

Table 6: Mechanisms of Antiepileptic Drugs[42]

Medication	Ion Channel	Excitatory Mechanism	Inhibitory Mechanism	Other
First generation				
Benzodiazepines			Enhances GABA	
Carbamazepine	Na^+, Ca^{2+} (L-type) blockade			
Ethosuximide	Ca^{2+} (T-type) blockade			
Phenobarbital	Increases Cl^- influx		Enhances and increases GABA	
Phenytoin	Na blockade			
Valproic acid	Na^+?/Ca^{2+} (L-type) blockade			
Second generation				
Felbamate	Na^+/Ca^+ blockade	Antagonizes NMDA receptors		
Gabapentin	Ca^{2+} (N-, P/Q-type)			

Lamotrigine	Na^+/Ca^{2+} (N-, P/Q-, R-, T-type) blockade		Increases GABA	
Levetiracetam	$K^+?/Ca^{2+}$ (N-type) blockade		Increases GABA	Binds to SV2A protein
Oxcarbazepine	Na^+/Ca^{2+} (N- and P-type)			
Pregabalin	Ca (N-, P/Q-type) blockade			
Rufinamide	Na^+ prolonged inactivation			
Tiagabine			Increases GABA	
Topiramate	Na^+ blockade	Antagonizes AMPA/kainate glutamate receptor	Enhances GABA	Inhibits carbonic anhydrase Enzyme
Vigabatrin			Increases GABA	
Zonisamide	Na^+/Ca^{2+} (N-, P-, T-type) blockade			Inhibits carbonic anhydrase Enzyme
Third generation				
Ezogabine	K^+ (enhances M-type current)			
Lacosamide	Increases slow inactivation of Na^+ channels			Binds to collapsin response mediator protein-2
Perampanel		Antagonizes AMPA glutamate receptor		

Ca^{2+} = calcium ion; GABA = γ-aminobutyric acid; K^+ = potassium ion; Na^+ = sodium ion; NMDA = N-methyl-D-aspartate *receptor; AMPA*= α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; SV2A = Synaptic vesicle glycoprotein 2A.

Epilepsy therapy almost always includes chronic use of one or more medications. As such, the treating epileptologist must be expert in selecting antiepileptic drugs (AEDs) and monitoring the patient's response to therapy over time. Epileptologists must be able to: (1) select antiseizure medications; (2) initiate and maintain AED dosing chronically;

(3) recognize and avoid dangerous and unnecessary AEDs; and (4) analyze response failure and AEDs with reference to serum levels.

Schematic representations of AED mechanisms at the synapse are presented in **Figure 10**.[43]

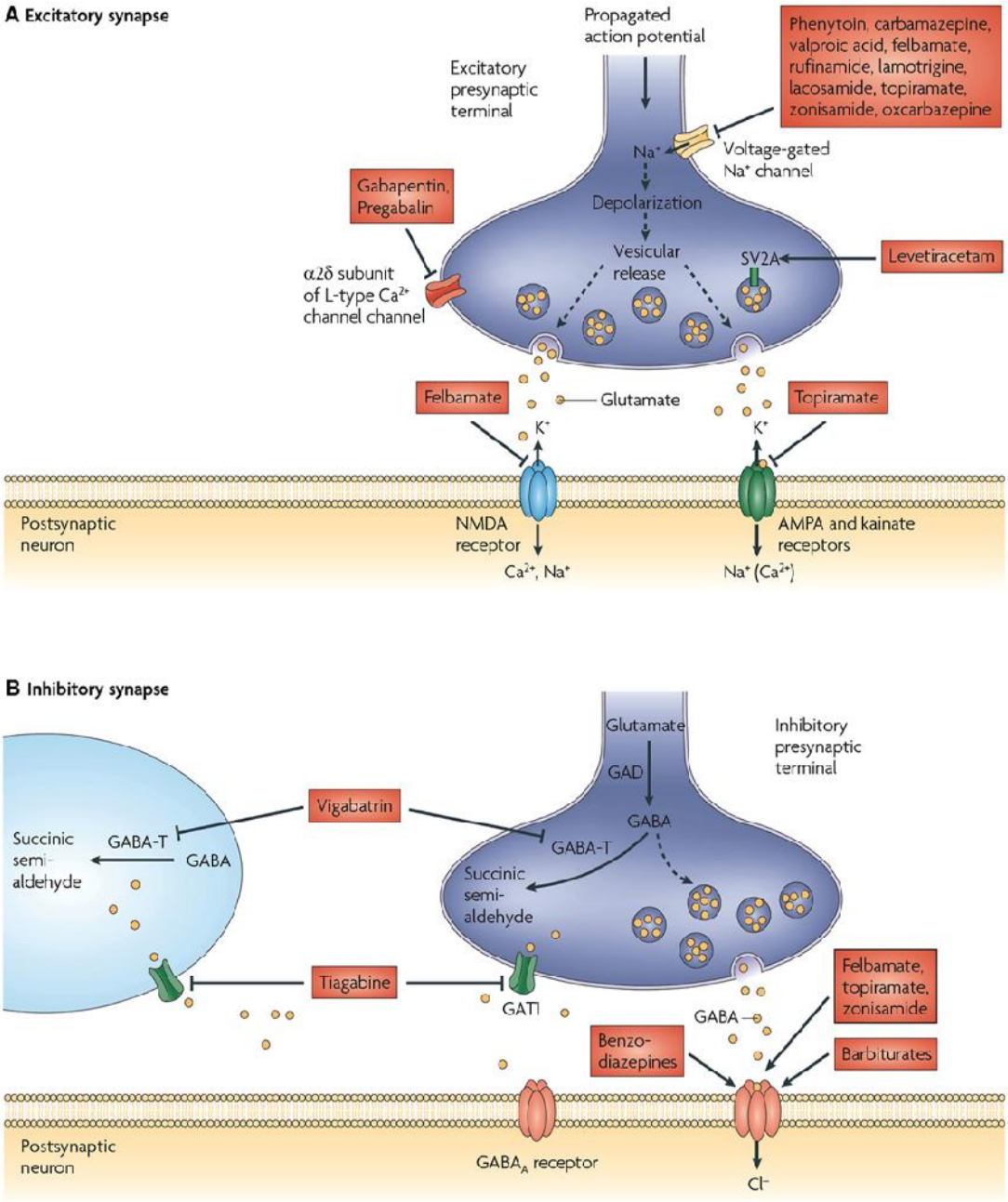


Figure 10: Excitatory (A) and inhibitory (B) synapse in the central nervous system and the sites of action of various anticonvulsants[43]

Pharmacokinetics

After a medication is administered, the body begins to redistribute, sequester, modify, and eliminate it. An understanding of pharmacokinetics allows the prescriber to select the best drug for a patient considering their current medications, comorbidities, and medication preferences. The ideal drug, whether used for epilepsy or any other condition, should be completely absorbed, minimally bind to proteins, distribute into the site of action, have minimal hepatic metabolism (and not interfere with the metabolism of other medications), and be eliminated by the kidney so that predictions about clearance can be made for an individual patient. Additionally, the dose and blood levels should have a linear relationship; for example, when a dose is doubled, the blood level doubles predictably. Many of the currently available AEDs lack these desired characteristics.

There are 4 principles of pharmacokinetics that should be considered when comparing and contrasting AEDs: absorption, distribution, metabolism, and elimination. Absorption is the movement of a drug molecule from the gut into blood to be circulated into other tissue(s).[44] Absorption is described by the Tmax (time to maximal peak blood levels) and the Cmax (the maximal concentration observed) in pharmacokinetic characterization studies. These parameters are important for determining bioavailability.[45] Medications may be passively absorbed, or transporters may be involved. One of the most clinically relevant examples of transporter involvement in absorption is gabapentin, whose absorption is limited by saturation of the L-amino acid transporter whereby the percentage of a dose absorbed decreases as the dose increases.[46] Adjustments in the

frequency and amount of gabapentin dose can be made, with smaller doses administered more frequently, to optimize the absorption. There are a number of other gut transporters, including P-glycoprotein and organic anion transporting polypeptide that may also play a role in drug interactions.[47] Changes in gut function, particularly rapid transit time or shortened intestines due to surgery, may radically reduce absorption. This can significantly impact the absorptions of medications delivered in an extended-release formulation, which require a sufficient amount of time in the gut or a specific environment, such as a certain pH, to be absorbed. Patients who have undergone a gastric bypass procedure may experience changes in their pharmacokinetics and require close monitoring, although the data in this area is currently limited.[48] After a drug is absorbed, it is distributed throughout the body. Distribution consists of 2 factors: how much drug is bound to proteins and how much drug is distributed into tissues.[49] If a medication is highly polar, it will primarily remain in the extracellular fluid. If a medication is lipophilic, it will more likely distribute into tissue compartments. The volume of distribution (Vd) is used to calculate loading doses:

$$\text{Loading dose} = (\text{concentration desired} - \text{baseline concentration}) \times \text{wt in kg} \times \text{Vd (L/kg)}$$

This equation is useful for estimating doses when a patient needs a new medication started rapidly or if their blood level is low and it needs to be increased. Metabolism is the enzymatic reaction(s) that occur(s) to a drug as the body is detoxifying itself. The majority of drug metabolism occurs in the liver. Phase I reactions are catalyzed by the cytochrome P-450 (CYP) family of enzymes, resulting in oxidized, reduced, or hydroxylated metabolites. Phase II reactions create polar metabolites that are more easily excreted into urine or bile. Either the parent drug or the metabolite from the phase I

reaction can be glucuronidated (phase II) for elimination.[50] Most medications (or their metabolites) are finally eliminated from the body via the kidneys into the urine. Glomerular filtration can be estimated with a variety of formulas using serum creatinine levels, including the Modification of Diet in Renal Disease study equation (MDRD), the Cockcroft-Gault equation, and most recently, the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-Epi).[51] The MDRD and CKD-Epi are used to stage kidney function.[52,53] The Cockcroft-Gault equation is frequently used for estimating kidney function when drug dose adjustments are required.[54] With the validation of new equations, some pharmaceutical companies have used methods other than the Cockcroft-Gault to renally adjust their products. As a result, each provider should refer to the prescribing information to verify if and how adjustments are made for declining kidney function. Human physiology changes over the life span and, as a result, the pharmacokinetics for a given patient on a given medication also will change over the life span. As patients age, increases in fat tissues and decreases in lean body mass impact volume of distribution.[55] Kidney function also declines with age.[56,57] Pregnancy in particular may alter hepatic function, with prominent effects on AED elimination that may require significant dosing changes to maintain effective serum concentrations of an agent such as lamotrigine.[58]

Drug-drug and drug-disease interactions

Drug interactions are frequently the result of an alteration of pharmacokinetics. In the case of AEDs, alterations in absorption and metabolism are the most common causes of drug interactions. Absorption interactions can be caused by 2 medications binding to each other, resulting in a chelate that cannot be absorbed. Tissues in the gastrointestinal tract

express CYP enzymes and P-glycoprotein, both of which can be inhibited or induced.[59,60] If CYP enzymes are inhibited, more medication can be absorbed from the gut, increasing exposure. If CYP enzymes are induced, more drug is metabolized in the gut, resulting in less absorption. P-glycoprotein is an efflux transporter located on the enterocytes that pumps medications back into the gut. Inhibition of P-glycoprotein results in an increase of absorption, while induction will result in a decrease of absorption. P-glycoprotein is also found in other tissues, including kidneys, pancreas, and the blood-brain barrier.[61] The mechanisms for metabolism (predominately in the liver) interactions are enzyme inhibition or enzyme induction. Hepatic inhibition occurs when the interacting drug competes for binding on the enzyme, resulting in decreased clearance of the affected drug. Hepatic induction occurs when there is an increase in the amount of enzyme available, resulting in increased clearance of the affected drug.[62] Older AEDs (phenobarbital, phenytoin, and carbamazepine) are some of the most common enzyme-inducing medications used, and they interact with a wide variety of medications.[63] Beyond pharmacokinetic drug interactions, some medications may lower seizure threshold and should be used with caution, including tramadol, bupropion (at higher doses and when using the immediate-release formulation), and clozapine.[64] Dalfampridine, the newly approved medication for improving walking distance for patients with multiple sclerosis, has been reported to cause seizures in patients taking the recommended doses.[65]

Calcium channel blockers and epilepsy

Clinically used calcium channel blockers act by blocking L-type and T-type of calcium channels which are present in various tissues including brain and they are

commonly prescribed for treatment of various clinical conditions such as hypertension, angina and cardiac arrhythmias.[66] In various experimental animal models, calcium channel blockers have been shown to exhibit the anti-inflammatory and antioxidant properties.[67] In addition calcium channel blockers have several advantages over the existing anti-epileptic drugs, such as no effect on hepatic microsomal enzymes, devoid of sedation, interference with quality of life and wide therapeutic range. They are even best suited for elderly individuals.

In the study Carried out by Umukoro S et al[68] to evaluate the anticonvulsant activity of two Calcium channel blockers, Verapamil and Nifedipine in Swiss albino mice. In the study, strychnine was administered intraperitonealy in the dose of 1mg/kg to induce convulsions. Both the calcium channel blockers prolonged the onset of seizures, when compared to controls ($p<0.05$).

Study done at university of Reggio Calabria,[69] Italy showed that calcium channel blockers like flunarizine, nifedipine, nicardipine, nimodipine, nitrendipine and diltiazem exhibited significant anticonvulsant activity in DBA/2 mice. In this study the convulsions were induced in experimental animals by auditory stimulation (109 dB). However, veapamil did not exhibit anticonvulsant activity in this study.

Brahmane et al[70] evaluated anti-convulsant activity of cinnarazine and nifedipine in mice using maximal electroshock and pentylenetetrazole models and showed that both the calcium channel blockers had significant anti- convulsant activity as compared to controls. It was also observed that both the calcium channel blockers significantly potentiated the effect of sodium valproate in both the models.

C. *IN VIVO* EXPERIMENTAL MODELS OF EPILEPSY

Introduction to Research on Experimental Models of Epilepsy

People who suffer this disease may have changes in their consciousness and motor abilities, among other things; also, due to the repetition of the seizures, neuropathological changes also take place, mainly in the hippocampus (Hp)[71] (**Figure 11**). The epileptic stimuli can directly activate interneurons, bipolar cells and basket, and these may inhibit projection cells. Also, the neurons projection can activate excitatory interneurons, which in turn act over projection neurons. Thus, changes in the function of one or more cells in a circuit can significantly affect neighboring or distant neurons. Other changes include formation of new synaptic connections as axons sprout. This sprouting of excitatory axons that establish many connections may result in an increase of the excitability of neurons necessary to produce repeated firing in the granule cells, characteristic of epileptic seizures.

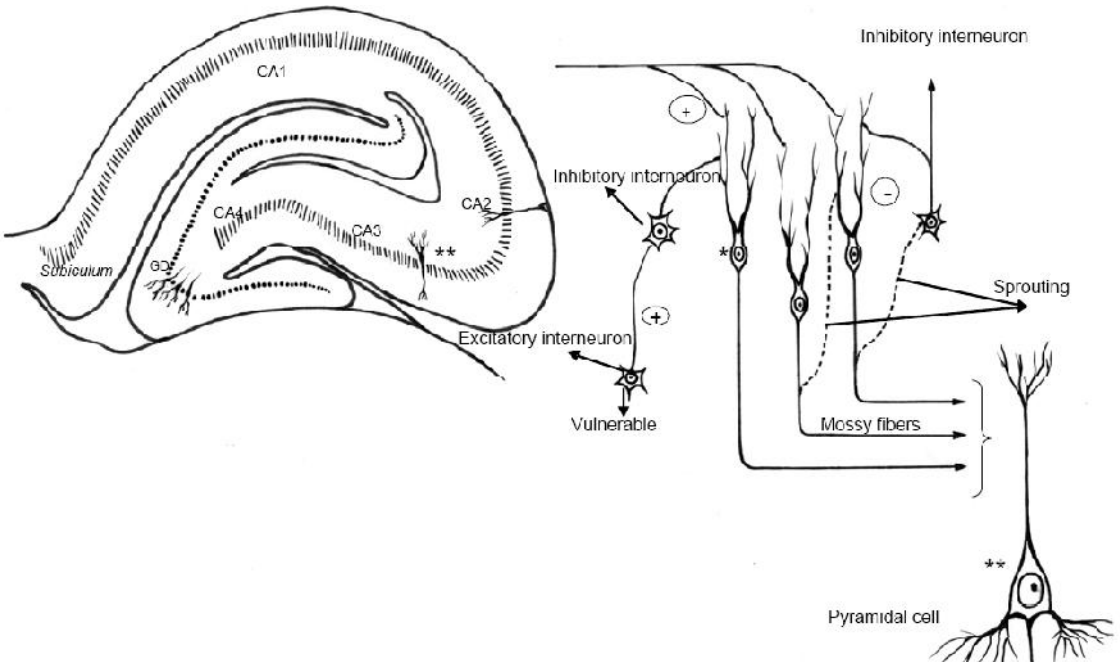


Figure 11: Neuropathological changes in hippocampus to epilepsy

Research on experimental models of epilepsy have helped to discover the most common physiopathological routes, and have led to believe that there is an imbalance in the central nervous system between the inhibitory GABAergic and excitatory glutamenergic neurotransmission (**Figure 12 and 13**). The subtypes of ionotropic glutamate receptors are AMPA kainite and NMDA. The activation of these receptors allows influx of ions. They differ from each other by cation permeability and by their different sensitivity to pharmacological agonists/antagonists of glutamate. All of glutamate ionotropic receptors are permeable to Na^+ and K^+, and it is the influx of Na^+ and K^+ exit through these channels that occurs in the cell membrane depolarization and action potential generation. NMDA receptors also facilitate the conductance Ca^{2+} which is blocked by ion Mg^{2+} in the resting state of the cell membrane, although during conditions of depolarization, the Mg^{2+} is displaced and the channel becomes permeable to Ca^{2+} influx. Finally ion tends to depolarize the cell as a factor that apparently contributes to neuronal damage induced by Ca^{2+}, in activation conditions neuronal excessive as that produced by epilepsy (**Figure 11**).

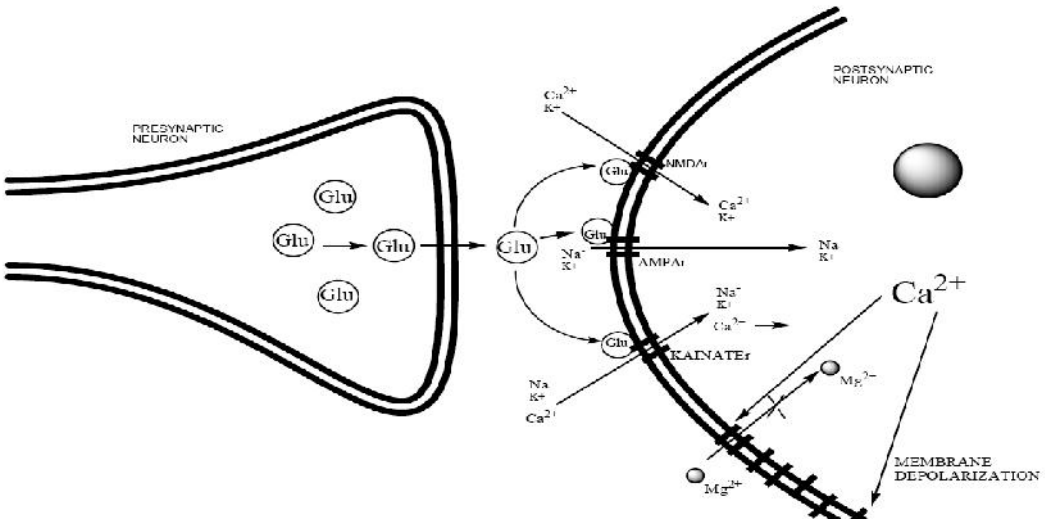


Figure 12: The subtypes of ionotropic glutamate receptors

GABAA receptors are permeable to Cl^- ions, as a result of activation. Influx of Cl- hyperpolarizes the membrane and inhibits the action potential. Based on the foregoing, the substances that are $GABA_A$ receptor agonists, such as barbiturates and benzodiazepines, suppress seizure activity. $GABA_B$ receptors are coupled to second messenger systems instead of Cl^- channels, and due to its location induce a reduction in presynaptic release of transmitters. Second messenger systems often result in opening of K^+ channels, inducing a hyperpolarizing current. In different models some $GABA_B$ receptor agonists exacerbate hyperexcitability and seizures (**Figure 13**).

Activation of glutamate receptors specifically N-metyl-D-aspartate (rNMDA), alpha amino-2,3-dihydroisoxazolepropanoic-5-methyl-3-oxo-4 acid (AMPA), a-kainate receptors and gamma-aminobutiric acid receptors ($rGABA_A$ and $rGABA_B$) are also involved.[72] Some studies also suggest that ion channels, in particular calcium (Ca^{2+}), sodium (Na^+) and potassium (K^+) channels, are involved in epilepsy.[73]

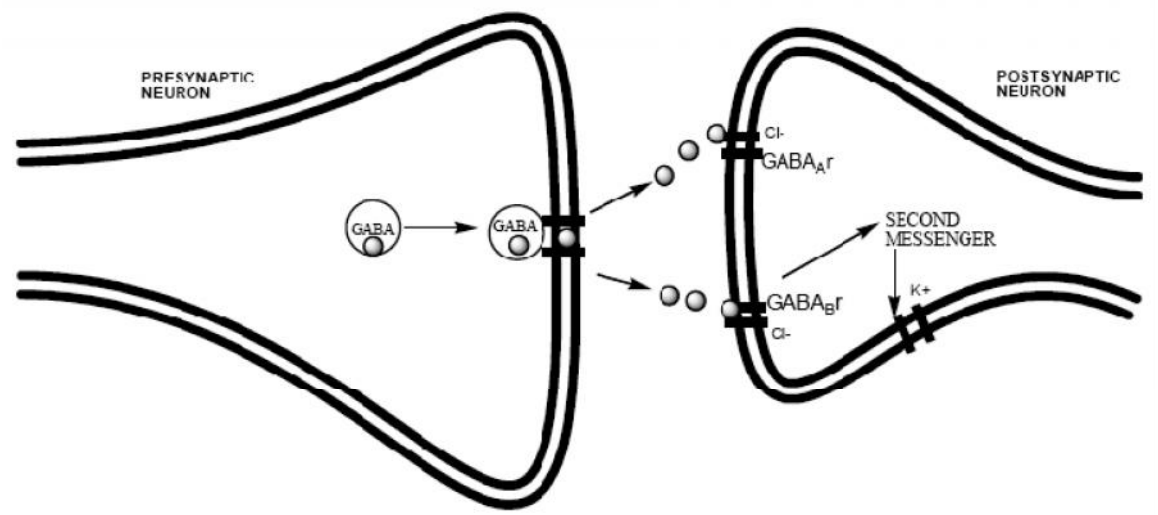


Figure 13 GABAA receptors

The incidence of epilepsy is over 1% of the total population. Worldwide, around 30% of patients do not respond to conventional drug treatments.[74] Consequently, several experimental models have been developed in which epileptic activity is simulated, and different treatments are tested to explain the neurochemical, neurophysiological, cellular and molecular mechanisms that control epileptic seizures. In addition, studies on these models have also been useful in the search for similarities of the mechanisms in human epilepsy. The different animal models can be classified as those induced by chemical convulsing agents, such as penicillin and cobalt, among others; models by electrical stimulation, such as kindling and electroshock, and genetic models, such as audiogenic seizures or the Papio papio baboon model.[75] These models have attempted to accurately explain the mechanisms that underlie this disease; however, the reason behind the existence of so many different types of epilepsy has not yet been found. In 1989, Fisher proposed an epileptogenic classification of models, which is similar to the classification used in patients: partial epilepsy, tonic-clonic convulsions, and status epilepticus (SE).[75] According to Fisher classification following are the main models of *in vivo* epileptogenesis currently known.[74]

Animal Models of ILAE against Epilepsy

1. *Simple Partial Seizure Models*
   - Cortically Implanted Metals
   - Aluminum, Cobalt, Zinc
2. *Complex Partial Seizure Model*
   - Kainic Acid Administration (KA)
   - Repetitive Electrical Stimulation (“Kindling”)
   - Administration of Tetanus Toxin
3. *Generalized Tonic Clonic Seizure Models*

- Maximum electroshock (MES)
- Pentylentetrazol (PTZ)
- Flutotyl

4. *Generalized Partial Seizure Models*

- Penicillin
- GABA
- Bicuculine

5. *Generalized Absence Seizure Models*

- Audiogenic seizures in mice.
- Genetic: Photosensitive *Papio papio*

6. *Status epilepticus*

- Pilocarpine

In recent years most of the knowledge and therapeutic progress in terms of epilepsy, has mainly come from laboratories and work with animals, mainly rats or mice. The varieties of experimental models of epilepsy have been the experimental basis for antiepileptic and pharmacological treatments. Therefore different animal models that have been developed in order to reproduce human epilepsy; some of the most commonly used models, and models that can only be developed *in vivo*. It is worth mentioning that they differ in their procedures: the electroshock model is developed using electrical stimulation, the kindling model also consists of applying electric stimuli, the difference being the place of administration. The kindling model has the important advantage of being able to study partial and generalized seizures. Within the models that use metals, it is known that the alumina cream model may have been the first experimental model developed to produce chronic epileptogenic foci; however, a number of factors limit its applicability. Hence, the alumina model has become less favored relative to other, more recently developed, techniques such as kindling. There are also models that induce

epilepsy by affinity with receptors, such as in the case of the KA model, or Bicuculine and GABA abstinence. However, they have limitations; for example the production of numerous lesions outside of the injection site. In addition, the overly high susceptibility of temporal lobe structures, limits the use of the KA model. The baboon model has been scarcely investigated, most likely due to the extensive limitations it has. The seizure model induced by penicillin in the cerebral cortex of experimental animals has been widely studied, and is one of the models that have best described several epilepsy mechanisms.

In present study we selected two epileptic rat models to evaluate the antiepileptic effect of clinically used calcium channel blockers and also to evaluate their ability to potentiate the antiepileptic effect of existing drugs. They are described in following text:

Electroshock Seizure (MES) Model

Electrical stimulation of the brain by placing electrodes on the cornea or ears has been used to induce motor convulsions that depend on the intensity of the stimulation. Tonic-clonic convulsions are produced by high electroshock currents, between 25 mA to 150 mA and 50 Hz with duration of 2 ms.[76,77] The intensity used depends on the animal, for example, in mice, a 45 mA current applied through a corneal electrode for 0.2 sec, as described elsewhere, is sufficient to produce a convulsive crisis.[78] Electrical stimulation current strengths tend to vary.[79] Large portions of the brain are stimulated with electroshock stimulation, causing generalized neural discharges.[80] Daily repetitive low current electroshock stimulation on a daily basis induces limbic kindling; evidence shows that responses are produced mainly in granule cells of the Hp, and also in the neocortex and pyriform cortex.[81] Also, electroshock-induced behavioral changes persist for at least

28 days.[82] Microinjection of GABA has an anti-convulsive effect in rats with seizures induced by electroshock.[83] This model has also helped to discover phenytoin, which reduced epileptic activity by 50%.[84] Different antiepileptic drugs were later tested using this model, such as carbamazepine and valproic acid.[85] Lamotrigin and oxcarbazepine inhibited seizures by 50% in this model.[86]

Pentylentetrazol (PTZ) Model

The Pentylentetrazol (PTZ) model for induction of epilepsy is considered to be similar to primary tonic-clonic generalized epilepsy in humans.[87] A 0.85% solution of PTZ in a volume of 0.01 mL/g body weight is administered by intracerebral injection,[88] or it can also be injected intraperitonially, in doses from 20 to 300 mg/kg. Mice are then observed for the presence or absence of a minimal clonic seizure of the forelimbs or vibrissae.[89] Episodes in each animal can be short, lasting 3-5 seconds. PTZ induced epilepsy is known to be generated and spread mainly through the Hp.[90] It has recently been reported that in rats treated with PTZ, there is an increase of axonal growth in the internal portion of the CA3 layer of the Hp.[91] It has also been reported that one the mechanisms that underlie epilepsy produced by PTZ, is increase of voltage in the voltage-gated potassium channel.[92] There is also a known relationship between the imbalance of the inhibitory and excitatory neurotransmission systems, and in the long run, a loss of inhibition mediated by GABA.[93] Specifically, PTZ blocks the $GABA_A$ receptor.[94] It is considered a GABA selective antagonist.[95] It is known that the expression of NMDA receptors undergoes subunit- and region-related changes in kindled seizures of rats induced by PTZ.[96] Carbamazepine is effective in inhibiting PTZ induced seizures.[97] Also, phenytoin and pentobarital have proven to have suppressing effects on the model.[98]

D. REVIEW OF DRUGS USED

Propylene glycol

Formula: CH_3 CHOH CH_2OH

Molecular weight: 76.10

A clear, colourless or almost odourless, viscous, hygroscopic liquid with a slight characteristic taste

Miscible with water, acetone, alcohol & chloroform, soluble in ether, immiscible with fixed oils but will dissolve in some essential oils.

Store in airtight containers

Propylene glycol is widely used in pharmaceutical manufacturing as solvent & vehicle especially for drugs miscible or insoluble in water.

Pentylenetetrazol (PTZ)

6,7,8,9 Tetrahydro-5H-Tetrazolo (1.5-9) azepine, Alpha, Beta cyclopentamethylene Tetrazole,

Formula: $C_6H_{10}N_4$

Molecular Weight = 138.2

Trade names: corozol, Leptazol, Pentamethazol, Pentazol, Cardiazol, Metrazol

Chemical Nature - Slightly Pungent, bitter crystals, melting point 57-60 degree. Freely soluble in water, in most organic solvents

Very stable, not easily attacked by other substances

LD_{50} in rats - 82 ±1 = 2 mg/ kg subcutaneously, 62 mg / kg intraperitoneally.

Pentylene tetrazol is a central & respiratory stimulant similar to doxapram hydrochloride. It has been used in respiratory depression but when respiratory stimulants are indicated other agents are generally preferred.

Phenytoin

Formula: $C_{15}H_{12}N_2O_2$

It is an anti-seizure medication.

Phenytoin is believed to protect against seizures by causing voltage-dependent block of voltage gated sodium channels. This blocks sustained high frequency repetitive firing of action potentials.

Phenytoin binds preferentially to the inactive form of the sodium channel. Because it takes time for the bound drug to dissociate from the inactive channel, there is a time dependent block of the channel. Since the fraction of inactive channels is increased by membrane depolarization as well as by repetitive firing, the binding to the inactive state by phenytoin sodium can produce voltage-dependent, use-dependent and time-dependent block of sodium-dependent action potentials.

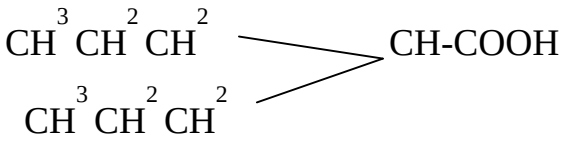
Phenytoin elimination kinetics shows mixed-order behaviour at therapeutic concentrations. A small increase in dose may lead to a large increase in drug concentration as elimination becomes saturated. The time to reach steady state is often longer than 2 weeks.

Common side effects include nausea, stomach pain, loss of appetite, poor coordination, increased hair growth, and enlargement of the gums. Potentially serious

side effects include sleepiness, self harm, liver problems, bone marrow suppression, low blood pressure, loss of balance or coordination and toxic epidermal necrolysis.

Sodium valproate

Valproic acid



Chemically it is n-dipropylacetic acid.

Formula: $C_8H_{16}O_2$

Valproic acid is strikingly different from phenytoin or ethosuximide in that it is effective in inhibiting seizures in a variety of models like phenytoin & carbamazepine. Valproate inhibits tonic hind limb extension in maximal electroshock seizures & kindled seizures at doses without toxicity. Like ethosuximide, valproic acid inhibits clonic motor seizures induced by pentylenetetrozole at subtoxic doses. Its efficacy in diverse models parallels against absence as well as partial & generalized tonic-clonic seizures in human beings.[99]

The action is similar to that of both phenytoin & carbamazepine & appears to be mediated by a prolonged recovery of voltage activated Na^+ Channels from inactivation. Another potential mechanism may involve metabolism of GABA. Although valproate has no effect on responses to GABA, it does increase the amount of GABA that can be recovered from brain after the drug is administered to animals.

Its extent of binding to plasma proteins is usually about 90%; Although concentration of valproate in CSF suggest equilibration with free drug in the blood, there is evidence for carrier mediated transport of valproate both into & out of the CSF.

The most common side effects are transient gastrointestinal symptoms, including anorexia, nausea & vomiting, effects on the CNS include sedation, ataxia, & tremor, rash, alopecia & stimulation of appetite have been observed occasionally. Elevation of hepatic enzymes, & a rare complication is fulminant hepatitis that is frequently fatal.[100]

Calcium channel blockers

Ca^{2+} ion is necessary for excitation-contraction coupling in both skeletal & smooth muscle. But in contrast to contractility of skeletal muscles the contractility of cardiac muscles & vascular muscle is highly dependent on the extracellular calcium concentration.

Calcium channel blockers interfere with the calcium entry in myocardial & vascular smooth muscle, thus decreasing availability of intracellular Ca^{2+}.

Calcium transport in the above sites involve three possible types of channel.

1. Voltage dependant channel – This is controlled by a gate that opens & closes in response to a voltage gradient. Calcium channel blockers close this gate & thus inhibit the entry of extracellular calcium ions.

2. Receptor operated channel – This channel is normally activated by α-adrenergic receptor agonist. The effect of calcium channel blockers may be due to blocking of the effects of NA on α –adrenoreceptors.

3. Sodium exchange channel – This is not of much relevance to calcium channel blockers.

1. Voltage dependant channel –

There are three voltage sensitive channels

Namely – ‘L’ type (Long lasting current)

‘T’ type (Transient current)

‘N’ type (Neuronal)

i. 'L' type – (Conductance is 25 ps,)

Activation threshold high inactivation rate is slow.

Location (1) Excitation - contraction

Function- coupling in cardiac & smooth muscle.

(2) SA, AV node – conductivity

(3) Neurotransmitter release.

Blockers – Nifedipine, diltiazem verapamil.

ii. ‘T’ type – (Transient current)

Conductance 8 ps, activation threshold – Low, inactivation rate fast

Location SA node- pace maker activity

Function -certain arteries constriction.

Blockers – flunarizine ,mibefradil, ethosuxemide.

iii. 'N' – type – conductance is 12 – 20 ps.

Activation threshold is high.

Inactivation rate medium.

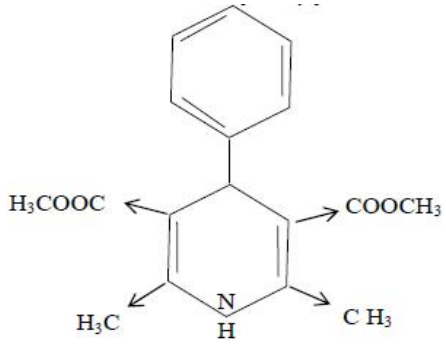
Location & Function } Only on neurones in CNS, sympathetic & mesenteric plexuses - transmitter release. Blocker is w – conotoxin.

Cardiovascular actions of calcium channel Blockers

1. Negative inotropic effect – These drugs depress the contractility of myocardium, & decrease cardiac work & myocardial oxygen consumption. These effects prove beneficial in treating angina of effort.
2. Antiarrythmic effect -These decrease the rate of discharge of the SA node, suppress ectopic pacemaker activity ,increase refractoriness of AV node & slow the conduction of a propagated impulse in myocardium. This prevents reentrant excitation. This effect plus improvement of cardiac ischaemia account for their potent antiarrythemic effect, verapamil & diltiazem are particularly useful in this respect.
3. Effect on coronary arteries – These drugs are more potent in dilating coronaries than nitroglycerine.
4. Effect on peripheral blood vessels – These drugs dilate vascular smooth muscle in systemic & pulmonary arterial circulation. Hence they have been used in treatment of systemic & pulmonary hypertension.
5. Antianginal property – These actions are due to improvement in the coronary blood flow. Reduce 0_2 demand of heart due to reduction in systemic vascular resistance & after load.

(a) **Nifedipine**

It is a dihydropyridine.



Structure

Its bioavailibility is about 40 -70%.

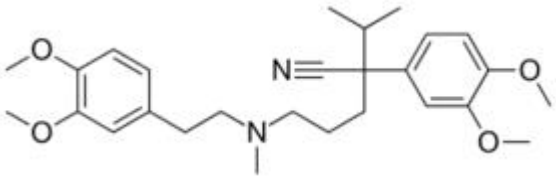
Plasma half life is 2-5 hrs.

It is a potent vasodilator, & has little inotropic activity.

It increases heart rate.

Its adverse effects are palpitation, hypotension, nausea & edema.

(b) Verapamil



Structure

Its bioavailibility is about 35.1%.

Plasma Half life: 2.8 hours

It is used for controlling ventricular rate in supraventricular tachycardia and migraine.

It works by relaxing the myocardium and blood vessels.

METHODOLOGY

MATERIALS AND METHODS

The study was conducted at Department of Pharmacology, B.L.D.E.U's Shri B.M. Patil Medical College Hospital and Research Center, Vijayapur.

Drugs used

1. **Pentylenetetrazol:** It was obtained from Sigma Aldrich Chemical Corporation Bangalore. The dose used was 70mg / kg given subcutaneously. It was dissolved in distilled water.

2. **Phenytoin (Diphenylhydantoin):** It was obtained from TORRENT PHARMACEUTICAL LIMITED, Doses used were 25 mg/kg & 12.5 mg/kg ,given intraperitoneally. The vehicle used was propylene glycol.

3. **Sodium valproate:** It was obtained from TORRENT PHARMACEUTICAL LIMITED, Doses used were 250 mg/kg & 125 mg/kg ,given intraperitoneally. The vehicle used was propylene glycol.

4. **Nifedipine:** This was obtained from TORRENT PHARMACEUTICAL LIMITED, Vehicle used was propylene glycol. The doses used were 5 mg/kg & 2.5 mg/kg given intraperitoneally.

5. **Verapamil:** This was obtained from TORRENT PHARMACEUTICAL LIMITED, Vehicle used was propylene glycol. The doses used were 20 mg/kg & 10 mg/kg given intraperitoneally.

Experimental Animals

Male albino rats weighing between 200-250gm, which were in bred in the central animal house B.L.D.E.U's Shri B.M. Patil Medical College, Vijayapur, were used to induce convulsions by both electroshock & pentylenetetrazol methods.

The above test animals were divided into two groups, one such group was subjected to electroshock & another was pentylenetetrazol.

The above test animals were divided into two groups. one such group was subjected to electroshock of 150 mA intensity for 0.2 seconds, through auricular electrodes, [covered in cotton wool & saline moistened). A majority of rats showed tonic flexion of fore & hind limbs with tail erection, tonic extension of both fore & hind limbs, clonus, stupor followed by post ictal depression & recovery. Only those rats showing the convulsive responses were used for experiment & divided into 6 groups of six each. Remaining group of rats were used for chemoshock (pentylenetetrazol) & divided into 6 groups of six each.

All the test animals were allowed food & water ad libitum both being withdrawn just prior to experimentation. All the test animals were subjected to further experiment of this study after 24 hours (to avoid any possible "kindling" effect) All the preparations were administered intraperitoneally except for pentylenetetrazol which was given subcutaneously.

Treatment Groups

Model 1: Maximal electro shock seizure (MES) Method 150mA for 0.2sec

Group I (Vehicle Control) as Propylene Glycol 0.5ml/100gm (i.p)

These animals constitute the control group for maximal electroshock (MES) method, which received 0.5ml propylene glycol /100gm of body weight, i.p. After an interval of 30 minutes they were subjected to electroshock [MES] stimulation of 150 mA for 0.2 seconds through transauricular electrodes by using techno-electro-convulsometer. The duration of different parameters were recorded.

Group II (Standard Positive Control) as Phenytoin 25mg/kg (i.p)

Six albino rats received 25 mg / kg of Phenytoin in propylene glycol, intraperitoneally and after an interval of 30 minutes they were subjected to maximal electroshock stimulation of 150 mA for 0.2 seconds. Duration of different parameters were recorded.

Group III (Nifedipine, 5mg/kg, i.p.)

Six albino rats received 5 mg / kg of nifedipine in propylene glycol intraperitoneally and after an interval of 30 minutes, they were subjected to maximal electroshock stimulation of 150 mA for 0.2 seconds. Duration of different parameters were recorded.

Group IV (Verapamil, 20mg/kg, i.p.)

Six albino rats received 20 mg / kg of Verapamil in propylene glycol intraperitoneally, and after an interval of 30 minutes, they were subjected to maximal electroshock stimulation of 150 mA for 0.2 seconds. The duration of different parameters were recorded.

Group V (Nifedipine, 2.5mg/kg, i.p. + Phenytoin 12.5mg/kg, i.p.)

This group of animals received 2.5mg/kg and Phenytoin 12.5mg/kg in propylene glycol, intraperitoneally and were subjected to maximal electroshock stimulation of 150 mA for 0.2 seconds after 30 minutes, and results were recorded.

Group VI (Verapamil 10mg/kg, i.p. + Phenytoin 12.5mg/kg, i.p.)

This group of animals received Verapamil 10mg/kg and Phenytoin 12.5mg/kg in propylene glycol, intraperitoneally and were subjected to maximal electroshock stimulation of 150 mA for 0.2 seconds after 30 minutes, and results were recorded.

Model 2: Pentylenetetrazol (PTZ) Method 70mg/kg, subcutaneously.

Group VII (Vehicle Control)

These animals constitute the control group for pentylenetetrazol method. Each rat of this group received 0.5ml propylene glycol /100gm of body weight, i.p. and after an interval of 30 minutes pentylenetetrazol (PTZ) 70 mg/kg body weight was injected subcutaneously. The duration of different parameters were recorded.

Group VIII (Sodium valproate, 250mg/kg, i.p.) as Standard Positive Control

Six albino rats received 250 mg / kg of sodium valproate in propylene glycol, intraperitonealy. After an interval of 30 minutes they were given pentylenetetrazol (PTZ) the dose of 70 mg/kg body weightt subcutaneously. The results were recorded.

Group IX (Nifedipine, 5mg/kg, i.p.)

These animals received the Nifedipine in propylene glycol at the dose of 5 mg / kg body weight intraperitonealy. After an interval of 30 minutes they were given pentylenetetrazol (PTZ) the dose of 70 mg/kg body weight subcutaneously. The duration of different parameters were recorded.

Group X (Verapamil, 20mg/kg, i.p.)

To this group of rats recieved Verapamil 20 mg / kg in propylene glycol, intraperitoneally. After 30 minutes pentylelenetetazol (PTZ) (70 mg / kg) was injected subcutaneously. The duration of different parameters were recorded.

Group XI (Nifedipine, 2.5mg/kg, i.p. + Sodium valproate 125mg/kg, i.p.)

This group of animals received 2.5 mg / kg and sodium valproate 125 mg / kg in propylene glycol, intraperitoneally. 30 minutes later pentylelenetetazol (PTZ) (70mg/kg) was injected subcutaneously. The duration of different parameters were recorded.

Group XII (Verapamil 10mg/kg, i.p. + Sodium valproate 125mg/kg, i.p.)

This group of animals received Verapamil 10 mg / kg and sodium valproate 125 mg / kg in propylene glycol, intraperitoneally. 30 minutes later pentylelenetetazol (PTZ) (70 mg / kg) was injected subcutaneously. The duration of different parameters were recorded.

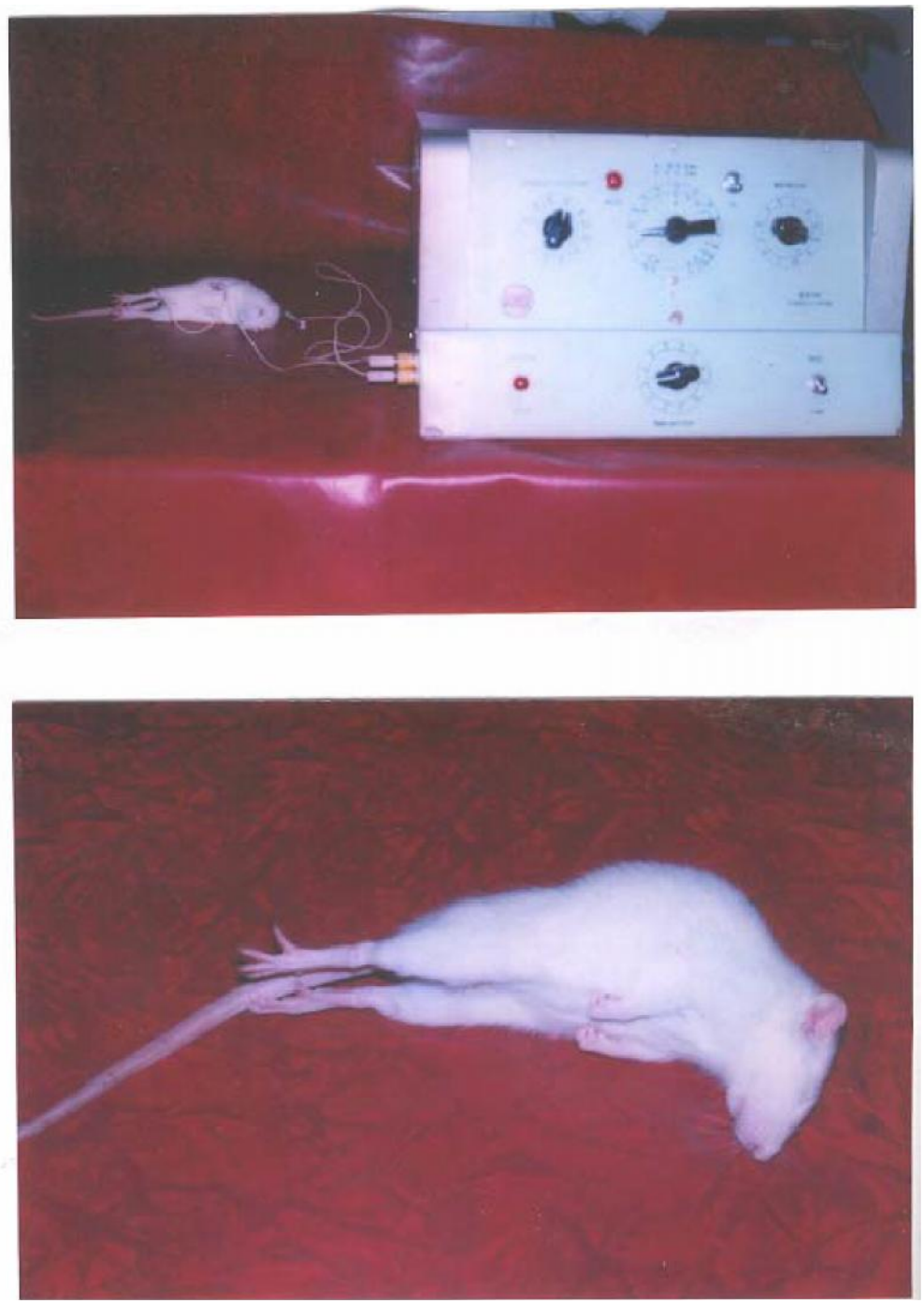


Figure 14: Untreated rat showing tonic extension of Hind Limbs on MES Stimulation (150mA for 0.2sec)

Experimental Methods

Maximal Electroshock (MES) method

Male albino rats weighing between 200-250 grams were subjected to maximal electroshock stimulation through transauricular electrodes with a current strength of 150 mA for 0.2 seconds. This result in seizures and various phases of seizures were noted and duration was recorded.

The parameters studied:

- The latency to convulse and duration of tonic convulsion (a tonic extension of the hind limb), the percentage seizure protection and percentage mortality were recorded.[101]
- Failure to extend the hind limbs to an angle with the trunk greater than 90 degree was defined as protection.[102]
- Formula for percentage protection : $\frac{\text{no. of animals with THLE absent} \times 100}{\text{Total no of animals in the group}}$

Pentylenetetrazol (PTZ) Method

Male albino rats weighing between 200-250 gramms received pentylenetetrazol (PTZ), 70 mg / kg body weight, injection subcutaneously. (in the scruff of neck through 27 gauge needle) and the resultant convulsions with its various phases to recovery or death were noted and duration of each parameters was recorded.

The parameters studied:

- The time taken before the onset of clonic convulsions (latency), the duration of clonic convulsions, the percentage of seizure protection and percentage mortality were recorded.[101]
- Abolition of clonic phase was considered as protection.[103]

Statistical Analysis

- All quantitative data was presented as Mean ± standard error of mean (SEM).
- Statistical analysis was done using the statistical software ‘‘Graphpad Prism 5’’ software version 5.00 San Diego California USA. (www.graphpad.com).
- The latency to convulse and duration of HLE were analysed by one way analysis of variance (ANOVA). For comparison between multiple group, one way ANOVA followed by post hoc Tukey’s test was performed.
- Analysis of the seizure protection and percentage mortality was done by Fisher’s exact test.
- The “*p*” value of <0.05 was considered as statistically significant.

RESULTS

RESULTS

In Maximal Electroshock (MES) Method the parameters like the latency to convulse, duration of tonic convulsion (hind limb tonic extension i.e HLE), the percentage seizure protection and the percentage mortality were recorded and results obtained in different groups represented in tables from 7 to 13 and figures from 15-18.

As seen in **Table 13,** Phenytoin group (Group II) shown complete abolition of convulsion, highly statistically significant decrease in duration of tonic hind limb extension, increase in seizure protection (83.33%) and any percentage mortality compared to control group ($p < 0.05$).

In only calcium channel blockers (Nifedipine and Verapamil) groups, Nifedipine group (Group III) and Verapamil group (Group IV) shown statistically non significant increase in latency to convulse and decrease in duration of tonic hind limb extension phase when compared to control group ($p < 0.05$). Whereas, in both these group statistically non significant increased percentage epilepsy protection and decreased percentage mortality was found when compared with control group (Group I).

Phenytoin and calcium channel blockers (Nifedipine and Verapamil) combination groups (Group V and VI) depicts statistically significant increase in latency to convulse and decrease in duration of HLE when compared to control group. In both these group statistically significant increased percentage epilepsy protection and decrease in percentage mortality was noted when compared to control group (Group I) ($p < 0.05$).

Tables 7-13 show results obtained in different groups with Maximal Electro Shock (MES) method

Table 7: Group I (Vehicle Control) Propylene Glycol 0.5ml/100gm (i.p)

Parameters	Serial No Of Animals							% Epilepsy Protection	% Mortality
	1	2	3	4	5	6	Mean ± SEM		
Latency to Convulsion (sec)	10	11	13	10	11	12	11.17 ± 0.89	0.00%	83.33%
Duration of HLE (sec)	25	25	20	32	35	28	27.50 ± 5.39		

HLE: Hind limb extensor tone
SEM: Standard error of mean
i.p.: Intraperitoneal

Table 8: Group II (Phenytoin 25mg/kg, i.p.) Standard Positive Control

Parameters	Serial No Of Animals							% Epilepsy Protection	% Mortality
	1	2	3	4	5	6	Mean ± SEM		
Latency to Convulsion (sec)	—	—	—	—	—	—	—	83.33%	0.00%
Duration of HLE (sec)	—	—	—	**20**	—	—	**3.33 ± 1.36**		

HLE: Hind limb extensor tone
SEM: Standard error of mean
i.p.: Intraperitoneal
— : Animals not developing convulsions.

Table 9: Group III (Nifedipine, 5mg/kg, i.p.)

Parameters	Serial No Of Animals							% Epilepsy Protection	% Mortality
	1	2	3	4	5	6	Mean ± SEM		
Latency to Convulsion (sec)	20	15	21	17	19	18	18.33 ± 2.16	33.33%	16.67%
Duration of HLE (sec)	22	28	—	27	25	—	17.33 ± 2.16		

HLE: Hind limb extensor tone
SEM: Standard error of mean
i.p.: Intraperitoneal
— : Animals not developing convulsions.

Table 10: Group IV (Verapamil, 20mg/kg, i.p.)

Parameters	Serial No Of Animals							% Epilepsy Protection	% Mortality
	1	2	3	4	5	6	Mean ± SEM		
Latency to Convulsion (sec)	15	18	10	11	20	11	14.17 ± 4.17	16.67%	33.33%
Duration of HLE (sec)	9	10	15	8	—	7	21.17 ± 1.77		

HLE: Hind limb extensor tone
SEM: Standard error of mean
i.p.: Intraperitoneal
— : Animals not developing convulsions.

Table 11: Group V (Nifedipine, 2.5mg/kg, i.p. + Phenytoin 12.5mg/kg, i.p.)

Parameters	Serial No Of Animals							% Epilepsy Protection	% Mortality
	1	2	3	4	5	6	Mean ± SEM		
Latency to Convulsion (sec)	33	29	40	37	25	28	32.00 ± 5.73	83.33%	0.00%
Duration of HLE (sec)	18	—	—	—	—	—	3.00 ± 1.22		

HLE: Hind limb extensor tone
SEM: Standard error of mean
i.p.: Intraperitoneal
— : Animals not developing convulsions.

Table 12: Group VI (Verapamil 10mg/kg, i.p. + Phenytoin 12.5mg/kg, i.p.)

Parameters	Serial No Of Animals							% Epilepsy Protection	% Mortality
	1	2	3	4	5	6	Mean ± SEM		
Latency to Convulsion (sec)	28	33	38	32	25	41	32.00 ± 5.73	66.67%	0.00%
Duration of HLE (sec)	—	—	—	—	21	—	3.50 ± 1.43		

HLE: Hind limb extensor tone
SEM: Standard error of mean
i.p.: Intraperitoneal
— : Animals not developing convulsions.

Table 13: Comparison of different parameters of various groups in MES Method

Experimental Groups	Parameters			
	Latency to Convulsion (sec)	**Duration of HLE (sec)**	**% Epilepsy Protection (%)**	**% Mortality (%)**
Group I (Vehicle Control, i.p)	11.17 ± 0.89	27.50 ± 5.39	0.00	83.33
Group II (Phenytoin, 25mg/kg, i.p)	—	3.33 ± 1.36*	83.33#	0.00#
Group III (Nifedipine, 5mg/kg, i.p)	18.33 ± 2.16	17.00±2.22	33.33	16.67
Group IV (Verapamil, 20mg/kg, i.p)	14.17 ± 4.17	21.17±1.77	16.67	33.33
Group V (Nifedipine, 2.5mg/kg, i.p + Phenytoin, 12.5mg/kg, i.p)	32.00 ± 5.73*	3.00 ± 1.22*	83.33#	0.00#
Group VI (Verapamil, 10mg/kg, i.p + Phenytoin, 12.5mg/kg, i.p)	32.83 ± 5.98*	3.50 ± 1.43*	66.67#	0.00#

HLE: Hind limb extensor tone; i.p :Intraperitoneal.
All the Latency to Convulsion and Duration of Convulsions values was quoted as the Mean ±Standard error of mean.
* $p < 0.05$ when compared to control group by one way ANOVA followed by post hoc Tukey's test.
# $p < 0.01$ when compared to control group by Fisher's exact test.
— : Groups not developing convulsions.

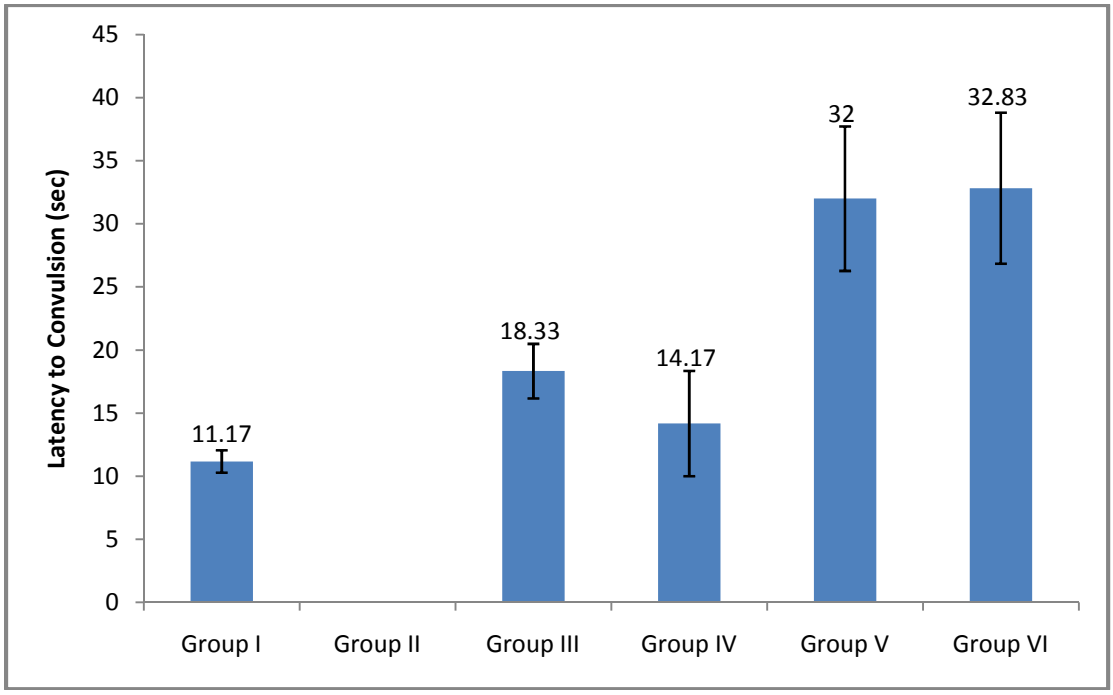


Figure 15: Mean ± SEM of latency to convulsions of experimental groups in MES method

Group I: (Vehicle Control, i.p)

Group II: (Phenytoin, 25mg/kg, i.p)

Group III: (Nifedipine, 5mg/kg, i.p)

Group IV: (Verapamil, 20mg/kg, i.p)

Group V: (Nifedipine, 2.5mg/kg, i.p + Phenytoin, 12.5mg/kg, i.p)

Group VI: (Verapamil, 10mg/kg, i.p + Phenytoin, 12.5mg/kg, i.p)

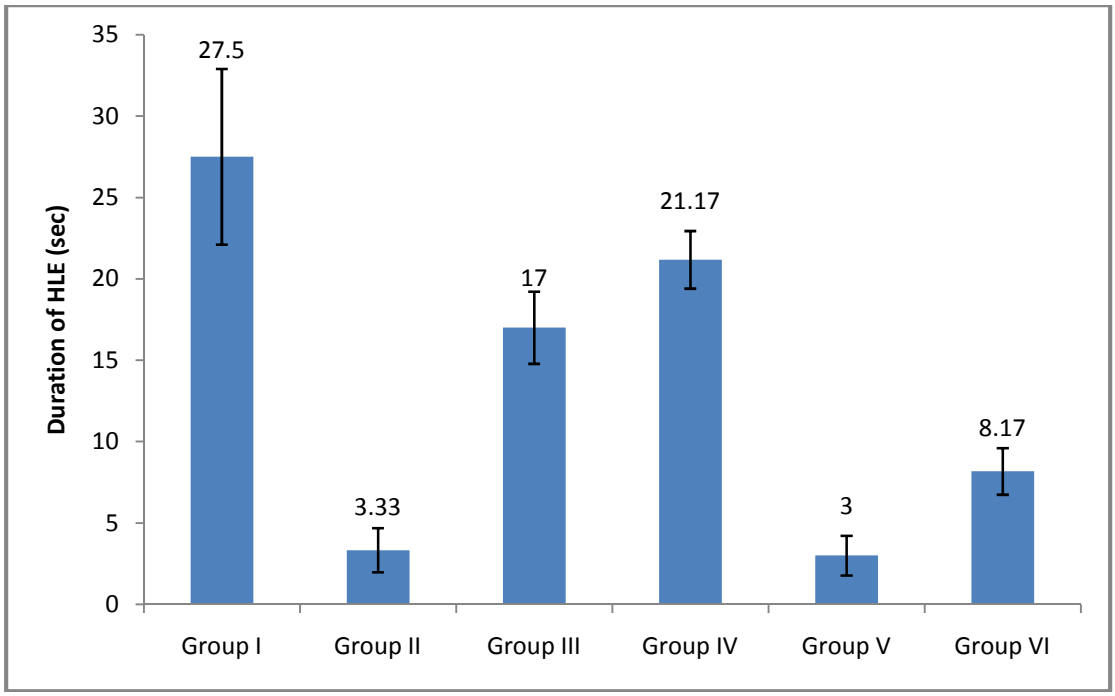


Figure 16: Mean ± SEM of duration of HLE of experimental groups in MES method

Group I: (Vehicle Control, i.p)

Group II: (Phenytoin, 25mg/kg, i.p)

Group III: (Nifedipine, 5mg/kg, i.p)

Group IV: (Verapamil, 20mg/kg, i.p)

Group V: (Nifedipine, 2.5mg/kg, i.p + Phenytoin, 12.5mg/kg, i.p)

Group VI: (Verapamil, 10mg/kg, i.p + Phenytoin, 12.5mg/kg, i.p)

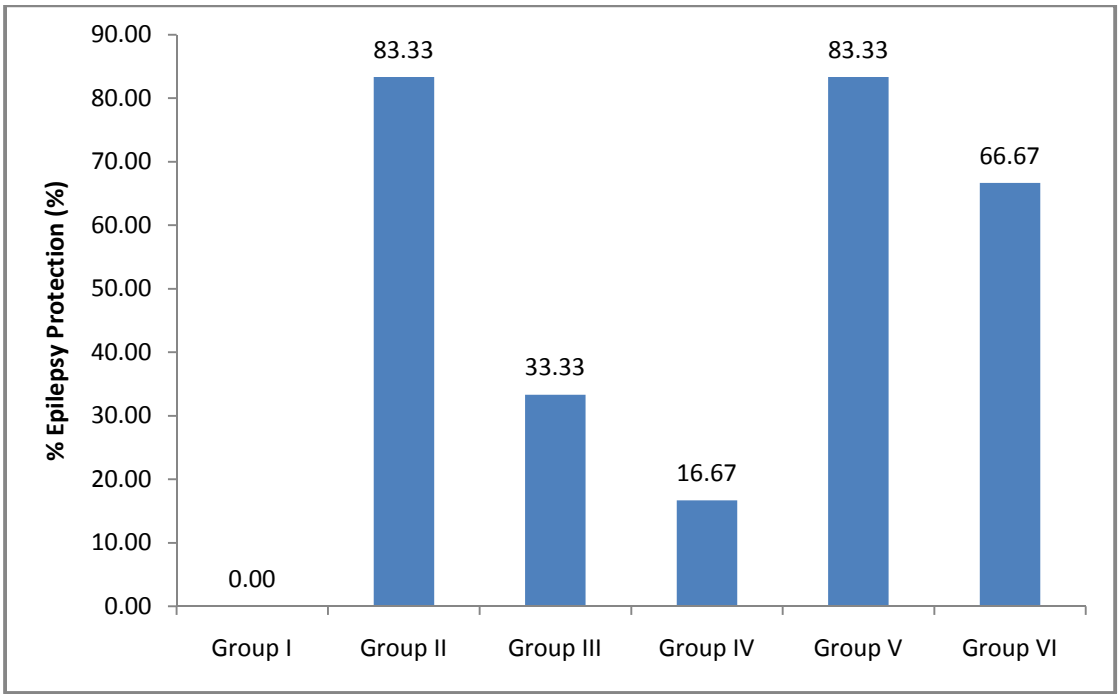


Figure 17: Mean ± SEM of percentage epilepsy protection of experimental groups in MES method

Group I: (Vehicle Control, i.p)

Group II: (Phenytoin, 25mg/kg, i.p)

Group III: (Nifedipine, 5mg/kg, i.p)

Group IV: (Verapamil, 20mg/kg, i.p)

Group V: (Nifedipine, 2.5mg/kg, i.p + Phenytoin, 12.5mg/kg, i.p)

Group VI: (Verapamil, 10mg/kg, i.p + Phenytoin, 12.5mg/kg, i.p)

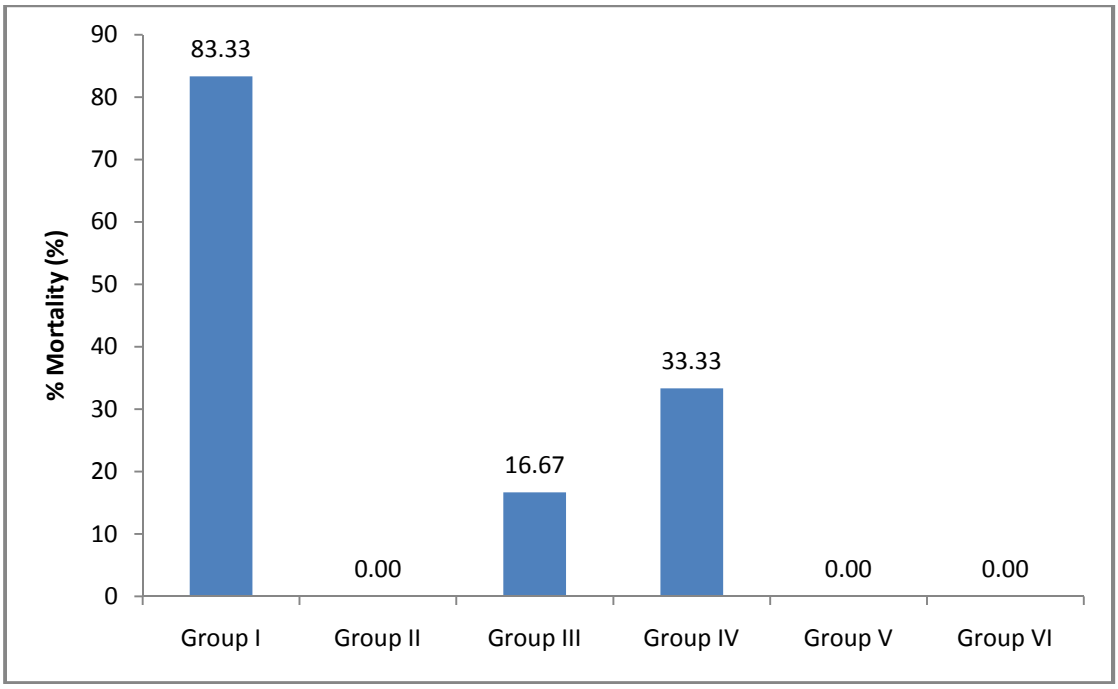


Figure 18: Mean ± SEM of percentage mortality of experimental groups in MES method

Group I: (Vehicle Control, i.p)

Group II: (Phenytoin, 25mg/kg, i.p)

Group III: (Nifedipine, 5mg/kg, i.p)

Group IV: (Verapamil, 20mg/kg, i.p)

Group V: (Nifedipine, 2.5mg/kg, i.p + Phenytoin, 12.5mg/kg, i.p)

Group VI: (Verapamil, 10mg/kg, i.p + Phenytoin, 12.5mg/kg, i.p)

In Pentylenetetrazol (PTZ) method the parameters like the latency to convulsion, duration of tonic convulsion, the percentage seizure protection and the percentage mortality were recorded and results obtained in different groups represented in tables from 14 to 20 and figures from 19-22.

As seen in **Table 20,** Sodium valproate group (Group VIII) shown complete abolition of convulsion, highly statistically significant decrease in duration of clonic convulsions, increase in seizure protection (83.33%) and any percentage mortality compared to control group (Group VII) ($p < 0.05$).

In only calcium channel blockers (Nifedipine and Verapamil) groups, Nifedipine group (Group IX) and Verapamil group (Group X) shown statistically significant increase ($p < 0.05$) in latency to convulsion and statistically non significant decrease in duration of clonic convulsion when compared to control group (Group VII). Whereas, in both these group statistically non significant increased percentage epilepsy protection and decreased percentage mortality was found when compared with control group (Group I).

Sodium valproate and calcium channel blockers (Nifedipine and Verapamil) combination groups (Group XI and XII) depicts statistically significant increase in latency to convulsions and decrease in duration of clonic convulsions when compared to control group (Group VII) ($p < 0.05$). In both these group statistically significant increased percentage epilepsy protection and decrease in percentage mortality was noted when compared to control group (Group I).

Tables 14-20 show results obtained in indifferent groups with Pentylenetetrazol (PTZ) method (70mg / kg, subcutaneous)

Table 14: Group VII (Vehicle Control) Propylene Glycol 0.5ml/100gm (i.p)

Parameters	Serial No Of Animals							% Epilepsy Protection	% Mortality
	1	2	3	4	5	6	Mean ± SEM		
Latency to Convulsion (sec)	258	325	306	290	410	382	328.50 ± 57.40	0.00%	100%
Duration of clonic convulsions (sec)	125	255	158	158	200	189	180.83 ± 44.93		

SEM: Standard error of mean
i.p.: Intraperitoneal

Table 15: Group VIII (Sodium valproate, 250mg/kg, i.p.) Standard Positive Control

Parameters	Serial No Of Animals							% Epilepsy Protection	% Mortality
	1	2	3	4	5	6	Mean ± SEM		
Latency to Convulsion (sec)	—	—	—	—	—	—	—	83.33%	0.00%
Duration of clonic convulsions (sec)	—	—	—	—	—	40	6.67 ± 16.67		

SEM: Standard error of mean
i.p.: Intraperitoneal
— : Animals not developing convulsions.

Table 16: Group IX (Nifedipine, 5mg/kg, i.p.)

Parameters	Serial No Of Animals							% Epilepsy Protection	% Mortality
	1	2	3	4	5	6	Mean ± SEM		
Latency to Convulsion (sec)	395	355	434	385	407	415	398.50 ± 27.17	33.33%	16.67%
Duration of clonic convulsions (sec)	155	165	—	198	150	—	111.33 ± 14.64		

SEM: Standard error of mean
i.p.: Intraperitoneal
— : Animals not developing convulsions.

Table 17: Group X (Verapamil, 20mg/kg, i.p.)

Parameters	Serial No Of Animals							% Epilepsy Protection	% Mortality
	1	2	3	4	5	6	Mean ± SEM		
Latency to Convulsion (sec)	383	350	408	390	416	345	382.00 ± 29.29	16.67%	33.33%
Duration of clonic convulsions (sec)	162	159	160	171	—	175	137.83 ± 67.83		

SEM: Standard error of mean
i.p.: Intraperitoneal
— : Animals not developing convulsions.

Table 18: Group XI (Nifedipine, 2.5mg/kg, i.p. + Sodium valproate 125mg/kg, i.p.)

Parameters	Serial No Of Animals							% Epilepsy Protection	% Mortality
	1	2	3	4	5	6	Mean ± SEM		
Latency to Convulsion (sec)	487	511	489	485	500	466	489.67 ± 15.17	83.33%	0.00%
Duration of clonic convulsions (sec)	—	41	—	—	—	—	6.83 ± 2.79		

SEM: Standard error of mean
i.p.: Intraperitoneal
— : Animals not developing convulsions.

Table 19: Group XII (Verapamil 10mg/kg, i.p. + Sodium valproate 125mg/kg, i.p.)

Parameters	Serial No Of Animals							%Epilepsy Protection	% Mortality
	1	2	3	4	5	6	Mean ± SEM		
Latency to Convulsion (sec)	466	509	436	498	474	481	477.33 ± 25.66	66.67%	0.00%
Duration of clonic convulsions (sec)	—	—	—	—	35	30	10.83 ± 2.81		

SEM: Standard error of mean
i.p.: Intraperitoneal
— : Animals not developing convulsions.

Table 20: Comparison of different parameters of various groups in PTZ Method

Experimental Groups	Parameters			
	Latency to Convulsion (sec)	**Duration of clonic convulsions (sec)**	**% Epilepsy Protection (%)**	**% Mortality (%)**
Group VII (Vehicle Control, i.p)	328.50 ± 57.40	180.83 ± 44.93	0.00	100
Group VIII (Sodium valproate 125mg/kg, i.p.)	—	6.67 ± 2.72*	83.33#	0.00#
Group IX (Nifedipine, 5mg/kg, i.p)	398.50 ± 27.17*	111.33 ± 14.64	33.33	16.67
Group X (Verapamil, 20mg/kg, i.p)	382.00±29.29*	137.83 ± 11.30	16.67	33.33
Group XI (Nifedipine, 2.5mg/kg, i.p + Sodium valproate 125mg/kg, i.p.)	489.67 ± 15.17*	6.83 ± 2.79*	83.33#	0.00#
Group XII (Verapamil, 10mg/kg, i.p + Sodium valproate 125mg/kg, i.p.)	477.33 ± 25.66*	10.83 ± 2.81*	66.67#	0.00#

i.p :Intraperitoneal.
All the Latency to Convulsion and Duration of Convulsions values was quoted as the Mean ±Standard error of mean.
* $p < 0.05$ when compared to control group by one way ANOVA followed by post hoc Tukey's test.
# $p < 0.01$ when compared to control group by Fisher's exact test.
— : Groups not developing convulsions.

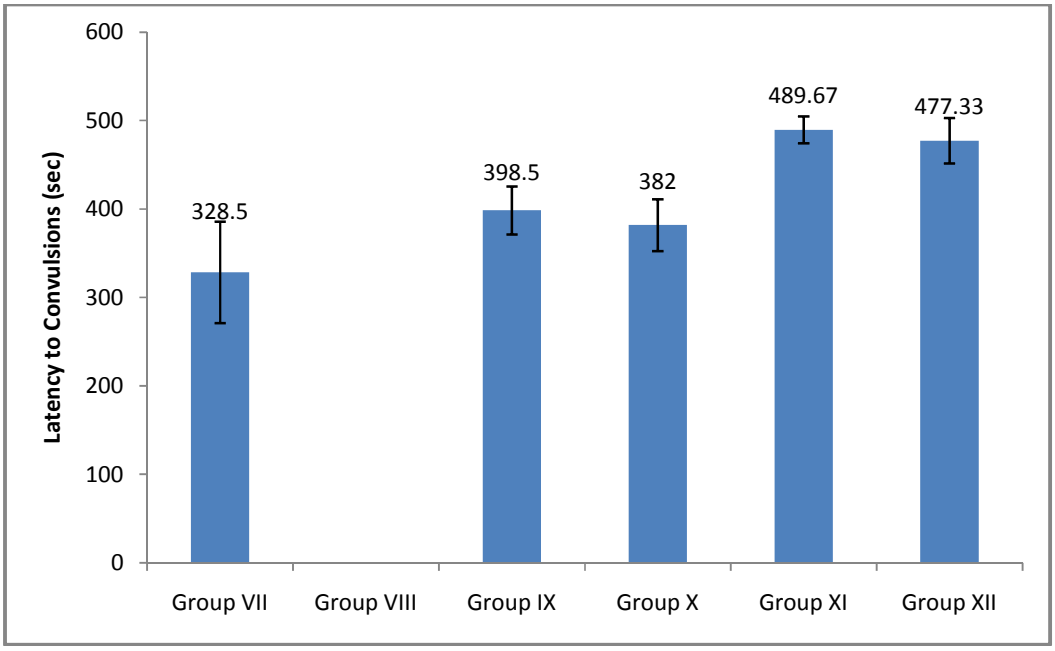


Figure 19: Mean ± SEM of Latency to Convulsion of experimental groups in PTZ method

Group VII: (Vehicle Control, i.p)

Group VIII: (Sodium valproate 125mg/kg, i.p.)

Group IX: (Nifedipine, 5mg/kg, i.p)

Group X: (Verapamil, 20mg/kg, i.p)

Group XI: (Nifedipine, 2.5mg/kg, i.p + Sodium valproate 125mg/kg, i.p.)

Group XII: (Verapamil, 10mg/kg, i.p + Sodium valproate 125mg/kg, i.p.)

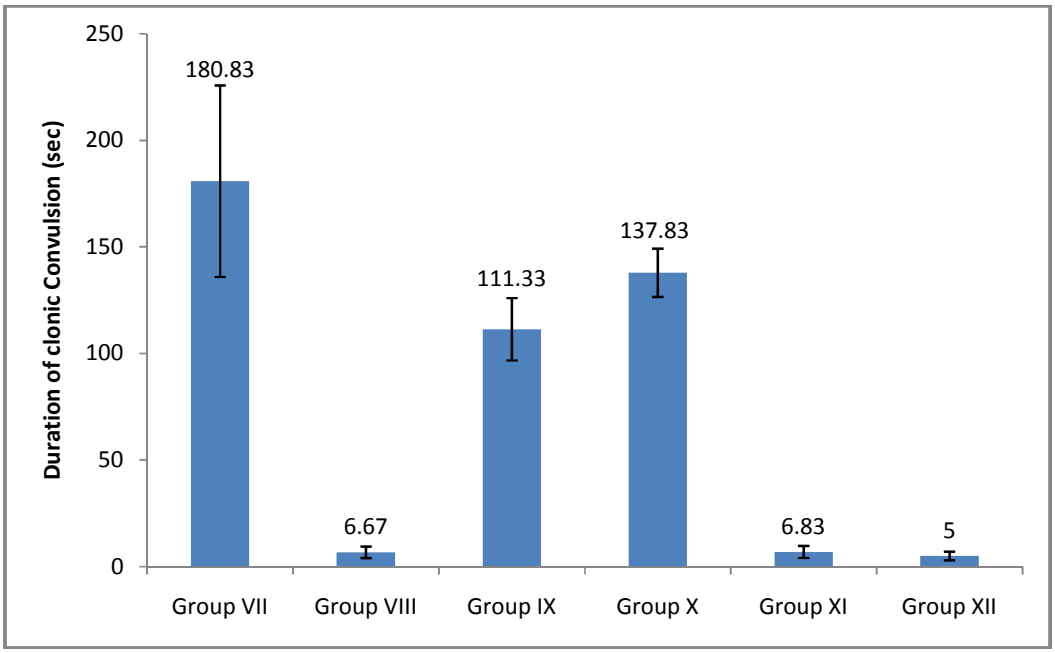


Figure 20: Mean ± SEM of duration of clonic convulsions of experimental groups in PTZ method

Group VII: (Vehicle Control, i.p)

Group VIII: (Sodium valproate 125mg/kg, i.p.)

Group IX: (Nifedipine, 5mg/kg, i.p)

Group X: (Verapamil, 20mg/kg, i.p)

Group XI: (Nifedipine, 2.5mg/kg, i.p + Sodium valproate 125mg/kg, i.p.)

Group XII: (Verapamil, 10mg/kg, i.p + Sodium valproate 125mg/kg, i.p.)

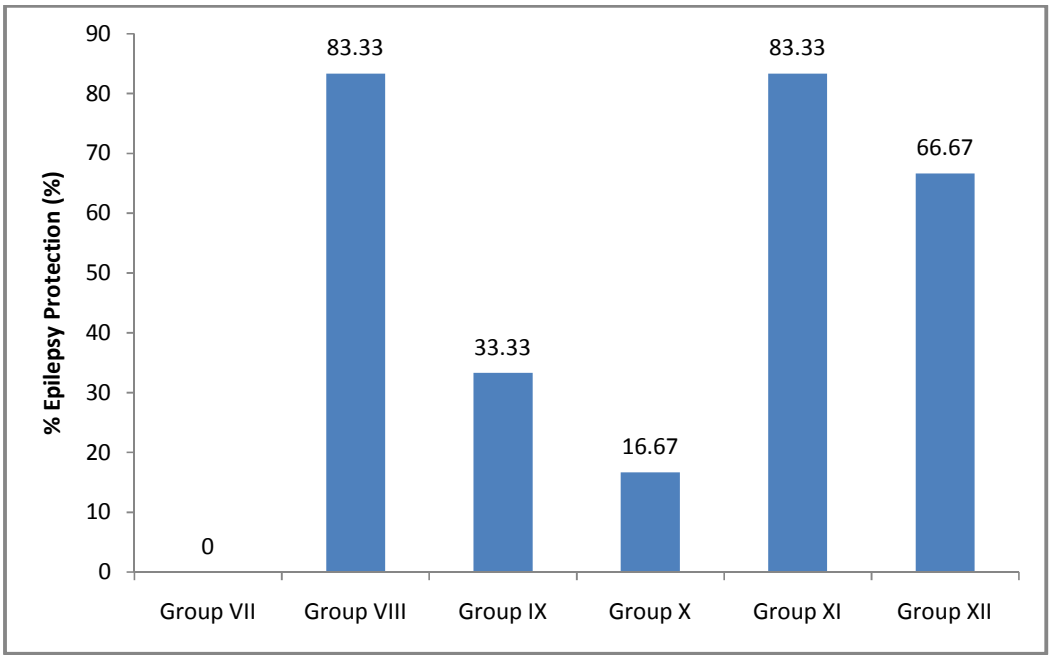


Figure 21: Mean ± SEM of percentage epilepsy protection of experimental groups in PTZ method

Group VII: (Vehicle Control, i.p)

Group VIII: (Sodium valproate 125mg/kg, i.p.)

Group IX: (Nifedipine, 5mg/kg, i.p)

Group X: (Verapamil, 20mg/kg, i.p)

Group XI: (Nifedipine, 2.5mg/kg, i.p + Sodium valproate 125mg/kg, i.p.)

Group XII: (Verapamil, 10mg/kg, i.p + Sodium valproate 125mg/kg, i.p.)

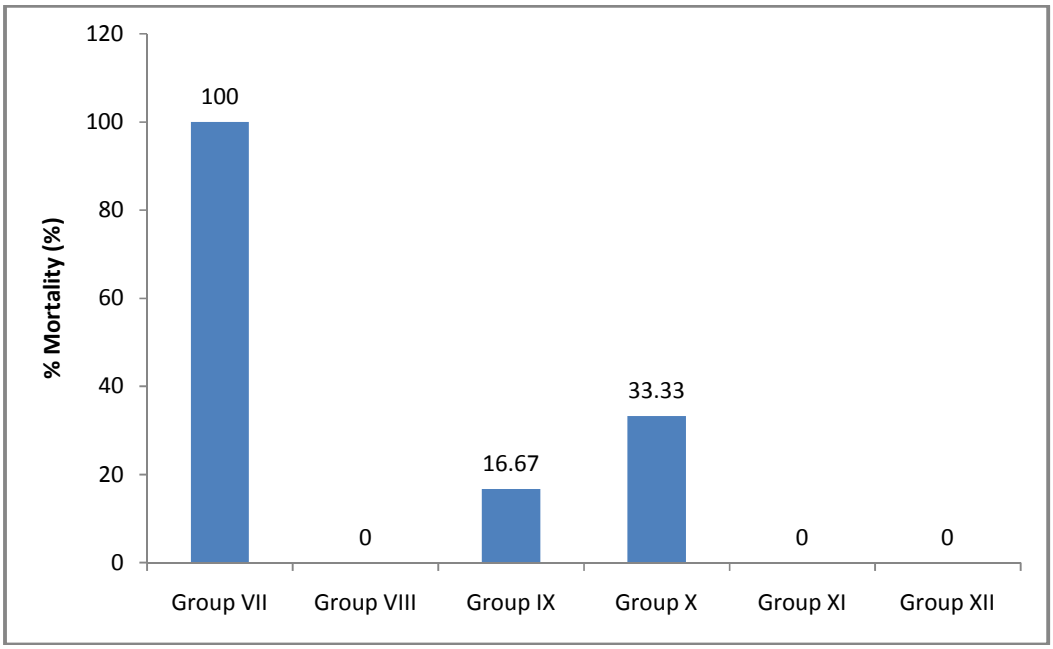


Figure 22: Mean ± SEM of percentage mortality of experimental groups in PTZ method

Group VII: (Vehicle Control, i.p)

Group VIII: (Sodium valproate 125mg/kg, i.p.)

Group IX: (Nifedipine, 5mg/kg, i.p)

Group X: (Verapamil, 20mg/kg, i.p)

Group XI: (Nifedipine, 2.5mg/kg, i.p + Sodium valproate 125mg/kg, i.p.)

Group XII: (Verapamil, 10mg/kg, i.p + Sodium valproate 125mg/kg, i.p.)

DISCUSSION

DISCUSSION

Despite advances in understanding of pathophysiology of epilepsy and improvement in its pharmacotherapy, the drug treatment remains far from satisfactory. In India, patients of epilepsy face social stigma, lack of education, resources constraints i.e. availability of neurospecialists, drugs and investigations etc results in their inadequate medical treatment.[104] In developing countries known etiology of epilepsy has been reported for less than 40% of cases, while industrialized countries have recorded a specific etiology for epilepsy in about 60-70% of cases.[105] In patients of epilepsy, antiepileptic drugs form main cornerstone of the treatment. Non compliance of drug treatment is important issue in drug discontinuation. It is commonly due to high incidence of side effects due to antiepileptic medications and ignorance.[106]

Calcium channel blockers are known to elicit direct negative inotropic effects on the heart' and tend to precipitate or aggravate the existing heart failure.

Calcium Channel blockers namely Nifedipine and Verapamil are screened for anticonvulsant activity by two most widely used models of epilepsy, viz. pentylenetetrazole model for absence seizures and the maximal electroshock model for generalized tonic-clonic seizures (GTCS). The test compounds showed anticonvulsant activity in both methods.

In PTZ induced seizure, our study finding showed, in only calcium channel blockers (Nifedipine and Verapamil) groups, Nifedipine group (Group IX) and Verapamil group (Group X) shown statistically significant increase ($p < 0.05$) in latency to convulsion and statistically non significant decrease in duration of clonic convulsion when compared to control group (Group VII). Whereas, in both these

group statistically non significant increased percentage epilepsy protection and decreased percentage mortality was found when compared with control group (Group I). Two out of six animals were protected against PTZ induced seizures in all the phases with Nifedipine (Table 16) and only one out of six animals was protected against PTZ induced seizures in all the phases with Verapamil (Table 17). Whereas, Sodium valproate and calcium channel blockers (Nifedipine and Verapamil) combination groups (Group XI and XII) results depicts statistically significant increase in latency to convulsions and decrease in duration of clonic convulsions when compared to control group (Group VII) ($p < 0.05$). In both these group statistically significant increased percentage epilepsy protection and decrease in percentage mortality was noted when compared to control group (Group VII). Five out of six animals were protected against PTZ induced seizures in all the phases with Nifedipine (Table 18) and similar four out of six animals were protected against PTZ induced seizures in all the phases with Verapamil (Table 19). Further, when we observed both these calcium channel blockers (Nifedipine and Verapamil) combination groups with Sodium valproate and standard control group (Group II) treated with only Sodium valproate showed almost same potential antiepileptic results on experimental animals.

In MES induced seizure, our study finding showed, in only calcium channel blockers (Nifedipine and Verapamil) groups, Nifedipine group (Group III) and Verapamil group (Group IV) shown statistically non significant increase in latency to convulsion and decrease in duration of HLE when compared to control group (Group I). Whereas, in both these group statistically non significant increased percentage epilepsy protection and decreased percentage mortality was found when compared with control group (Group I). Two out of six animals were protected against MES

induced seizures in all the phases with Nifedipine (Table 9) and only one out of six animals was protected against MES induced seizures in all the phases with Verapamil (Table 10). Whereas, Sodium valproate and calcium channel blockers (Nifedipine and Verapamil) combination groups (Group V and VI) results depicts statistically significant increase in latency to convulsions and decrease in duration of clonic convulsions when compared to control group (Group I) ($p < 0.05$). In both these group statistically significant increased percentage epilepsy protection and decrease in percentage mortality was noted when compared to control group (Group I). Five out of six animals were protected against MES induced seizures in all the phases with Nifedipine (Table 11) and similarly four out of six animals were protected against MES induced seizures in all the phases with Verapamil (Table 12). Further, when we observed both these calcium channel blockers (Nifedipine and Verapamil) combination groups with Phenytoin and standard control group (Group II) treated with only Phenytoin showed almost same potential antiepileptic results on experimental animals.

Various experimental studies have reported anticonvulsant potential of Nifedipine and Verapamil in combination with existing antiepileptic drugs or alone.

The study carried out by Umukoro S et al[67] to evaluate the anticonvulsant activity of two calcium channel blockers, Verapamil and Nifedipine in Swiss albino mice. Both the calcium channel blockers prolonged the onset of seizures, when compared to controls ($p<0.05$). Another study[68] showed that calcium channel blockers exhibited significant anticonvulsant activity in DBA/2 mice.

Brahmane et al[70] evaluated anticonvulsant activity of nifedipine in mice using maximal electroshock and pentylenetetrazole models and showed that the calcium channel blockers had significant anticonvulsant activity as compared to controls. It

was also observed that both the calcium channel blockers significantly potentiated the effect of sodium valproate in both the models.

El-Azab MF, Moustafa YM[107] demonstrated that the three calcium channel blockers afforded a protection against sub-convulsive doses of PTZ. Their protective effects were comparable to that exerted by the standard antiepileptic drug, sodium valproate. The anticonvulsant activity of sodium valproate was further enhanced by its combination with diltiazem. Also, PTZ-kindling reduced pain-threshold as evaluated by hot plate analgesimeter and acetic acid-induced writhing test. Although the repeated administration of sodium valproate significantly increased pain-threshold in kindled mice, it was not able to normalize it. Similar results were obtained with diltiazem and nifedipine. Interestingly, combination of diltiazem or nifedipine with sodium valproate elicited the most profound antinociceptive effect in kindled mice.

Abdel-Rahmanm et al[108] revealed that the i.p. injection of nifedipine into mice produced significant increase in the seizure onset of KA action and the MES of mice as well as, the percent protection of mice to a value of 25%. In addition, our results indicated that the single i.p injection of sodium valproate elicited significant increases in the onset of KA-induced seizures, the MES of mice as well the KA-induced mortality by a factor of 50%. The combined administration of nifedipine and sodium valproate was found to protect all animals from KA-induced death. This 100% protection was associated with 4-fold increase in the onset of KA-induced seizures. Measurement of the serum and brain electrolytes in mice receiving the combination of nifedipine and sodium valproate demonstrated that this combination caused significant diminution of brain Na^+ and Ca^{2+} ions and a rise in brain K^+ ions. It may be the ability of nifedipine to reduce brain Na^+ and Ca^{2+} concentration which is the

factor responsible for the additive anticonvulsant effect recorded after the combined administration of nifedipine and sodium valproate.

Wari UG[109] demonstrated that both calcium channel blockers (flunarizine and nifedipine) afford protection against convulsions induced in both models, and flunarizine affords higher degree of protection than nifedipine, with its efficacy almost approaching that of sodium valproate.

Sahadevan and Rema[110] suggested pretreatment with nifedipine, 5mg/kg reduced the duration of tonic extensor phase and 30% ofanimals were protected from the occurrence of convulsions. The maximum effect seen with 5mg/kg of nifedipine.

Khobragade et al demonstrated[111] calcium channel blocker (Flunarizine) was found to reduce the durations of tonic extensor phase, convulsion and post-ictal phase in a statistically significant way in the MES model; and while used in combination with phenytoin, the results were statistically better than both the drugs given individually. In the PTZ model, flunarizine enhanced the latency of onset of seizures and decreased the durations of convulsions and post-ictal phase in a statistically significant way; and the combination of flunarizine and valproate was statistically significant than either drug alone. Flunarizine has shown potential as an individual antiepileptic drug as well as a useful add-on therapy with standard antiepileptic drugs like phenytoin and valproate in both the models of epilepsy.

Desai et al[112] observed Pentylenetetrazole (PTZ)-induced convulsions and the maximal electroshock (MES) seizure test were employed to study the anticonvulsant effects of nifedipine (2, 3.5 and 5 mg kg-1), flunarizine (10, 20 and 40 mg kg-1) and diltiazem (10, 15 and 30 mg kg-1). Nifedipine and flunarizine prolonged the latent period and reduced the mean duration of PTZ induced seizures. They also reduced the severity of convulsions and the number of deaths due to PTZ significantly. Nifedipine

was more potent in this regard ($P < 0.01$). All these drugs prolonged the latent period and reduced the duration of tonic extensor phase of MES seizures in a significant manner. Flunarizine was most potent in this test. Complete protection from tonic extensor phase was observed in 10-50% animals pretreated with nifedipine and flunarizine in a dose dependent manner. The response of diltiazem was weak in both these tests. It is concluded that all three calcium channel blockers possess an important but different anticonvulsant effect and their significant clinical use can be made while keeping in view the characteristics of their pharmacological action.

Rodger and Pleuvry[113] studied the anticonvulsant effect of flunarizine has been compared to that of nifedipine in the pentylenetetrazole (PTZ)-induced seizure incidence/latency test and the PTZ seizure threshold test in mice. Nifedipine was found to have anticonvulsant activity in both models but flunarizine only had an anticonvulsant effect in the PTZ seizure incidence/latency test. Interactions with commonly used anti-epileptic drugs were also examined. In both models, the anticonvulsant effects of small doses or carbamazepine and phenytoin were enhanced by flunarizine and the effects of small doses of ethosuximide were enhanced by nifedipine. The effects of large doses of the anti-epileptic drugs were not further enhanced by either flunarizine or nifedipine. It is possible that these findings in mice are relevant to the variability of the responses to calcium antagonists as add-on therapy for epilepsy in man.

Our study is in agreement with the findings of the authors cited above. However, this is dearth of information on this subject. It needs further experimental studies as these preliminary reports are in rodents only. The study should be carried out in higher animals like canines and subhuman primates.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Epilepsy is a common and a complex neurological disorder that affects health and quality of life. At present forty different types of antiepileptic drugs (AED) are available. However, existing drugs can control seizures in only 60%–70% cases of epilepsy. The remaining, constitute the drug resistant epilepsy (DRE) group. Hence there is a constant search of a new effective antiepileptic agent.

In recent years there is an increasing evidence of neuroprotective effect of calcium channel blockers i.e. Nifedipine and Verapamil. There are several preclinical and clinical studies indicating neuroprotective potential of calcium channel blockers. Calcium ion is a regulator of metabolic pathways and serves important functions as a second messenger. Calcium ion influx occurs by voltage dependent and/or receptor-operated calcium channels. A calcium ion flux into the intracellular space represents the first stage of epileptic neuronal events. The initiation of epileptogenic activity in the neuron is thought to involve the normal phenomenon known as “intrinsic burst firing” that is activated by an inward calcium ion current. It was demonstrated that calcium ion flux into the pre-synaptic terminal is an important factor for neurotransmission. The blockade of different types of calcium ion channels was proposed as a possible mechanism of action of standard antiepileptic drugs. Furthermore, epileptic depolarizations of neurons were found to be depressed by calcium ion channel blockers. Comorbidities of epilepsy comprise some pain disorders, including acute nociceptive pain, therefore, antiepileptic drugs can prove efficacy in the management of this kind of pain albeit with several adverse reactions.

On this background we have evaluated the modulatory effects of calcium channel blockers on the anticonvulsant and antinociceptive effects of standard antiepileptic

drugs like Phenytoin and Sodium valproate in pentylenetetrazole (PTZ) and Maximal Electroshock (MES) albino rats.

Nifedipine showed anticonvulsant activity in both pentylenetetrazole induced and maximal electroshock induced seizures by increasing the latency for convulsion and reducing mean duration of convulsions.

Verapamil also showed anticonvulsant activity in both pentylenetetrazole induced and maximal electroshock induced seizures by increasing the latency for convulsion and reducing mean duration of convulsions.

The mechanism of action of calcium channel blockers is not precisely known, however, anti-inflammatory, antioxidant, antiapoptosis, etc. are proposed mechanism for their action. Further experimental and clinical studies are needed to confirm it.

Conclusions were drawn from this study as follows,

1. Alone Nifedipine and Verapamil have shown moderate antiepileptic effect against pentylenetetrazole and maximal electroshock induced seizures.
2. Combination of Nifedipine and Verapamil with existing antiepileptic drugs (Phenytoin and Sodium valproate) has shown potential antiepileptic effect to potentiate the anticonvulsant activity against pentylenetetrazole and maximal electroshock induced seizures.
3. Therapeutically, this enhancing profile for calcium channel blockers fosters a safer and more effective drug-combination regimen than existing antiepileptic drugs alone.

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ANNEXURES

ANOVA for comparing mean study parameter among study groups in MES method

Groups	**Mean study parameter**	*P-value*
Group I		
Group II		
Group III		
Group IV		
Group V		
Group VI		

Multiple comparison (ANOVA) followed by post hoc Tukey's test of mean study parameter among study groups

Multiple comparison		**Mean difference of study parameter**	**p value**
Group I	Group II		
	Group III		
	Group IV		
	Group V		
	Group VI		
Group II	Group III		
	Group IV		
	Group V		
	Group VI		
Group III	Group IV		
	Group V		
	Group VI		
Group IV	Group V		
	Group VI		
Group V	Group VI		

ANOVA for comparing mean study parameter among study groups in PTZ method

Groups	**Mean study parameter**	*P-value*
Group VII		
Group VIII		
Group IX		
Group X		
Group XI		
Group XII		

Multiple comparison (ANOVA) followed by post hoc Tukey's test of mean study parameter among study groups

Multiple comparison		**Mean difference of study parameter**	**p value**
Group VII	Group VIII		
	Group IX		
	Group X		
	Group XI		
	Group XII		
Group VIII	Group IX		
	Group X		
	Group XI		
	Group XII		
Group IX	Group X		
	Group XI		
	Group XII		
Group X	Group XI		
	Group XII		
Group XI	Group XII		

The "*p*" value of <0.05 was considered as statistically significant

Fisher Exact test for analysis of the seizure protection and percentage mortality

The Fisher Exact test formula:

$$\mathbf{P = ((a + b)! (c + d)! (a + c)! (b + d)!) / a ! b ! c ! d ! N !}$$

In this formula, the ‘a,’ ‘b,’ ‘c’ and ‘d’ are the individual frequencies of the 2X2 contingency table, and ‘N’ is the total frequency.

The Fisher Exact test uses this formula to obtain the probability of the combination of the frequencies that are actually obtained.

It also involves the finding of the probability of every possible combination which indicates more evidence of association.

The “*p*” value of <0.05 was considered as statistically significant