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Review Article

MODELS OF EPILEPSY USED IN ANTIEPILEPTIC DRUG DISCOVERY: A REVIEW

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ABSTRACT

This article describes various experimental models of seizure and epilepsy. Epilepsy is characterised by recurrent unprovoked seizures. Antiepileptic drug discovery in animal models starts with the assumption that the experimental seizure model mimics human seizure. Hence a drug which suppresses ictogenesis or inhibits epileptogenesis in animal model is a potential antiepileptic drug for human and it needs further investigation. Phenytoin and Carbamazepine were identified with the help of relatively simple models like Maximal electroshock seizure and the pentylenetetrazole test. Lots of drugs were discovered with the help of these models but a big portion of patients still remains resistant to the available antiepileptic drugs. Again these simple seizure models are increasingly being questioned, are they providing us same type of drugs with same kind of mechanism of action? This question brings the importance of newer animal models that target epileptogenesis, pharmacoresistant epilepsy and models which mimic human epilepsy more closely. There is increased concern on agents for epilepsy disease modification and prevention. To solve these unmet needs, the research scientist must have a thorough knowledge of available animal models of epilepsy so that he can pick up the best model for his research. In this article, we are reviewing the diversity of animal models of epilepsy and their implications in antiepileptic drug discovery.

Keywords: Animal models, Epilepsy, Pharmacoresistant, Antiepileptogenic.

INTRODUCTION

Epilepsy is a disease of the brain characterised by at least two unprovoked (or reflex) seizures occurring more than 24 hours apart [1].In epilepsy, there is a predisposition to generate epileptic seizures [2]. Seizure occurs due to abnormal excessive or synchronous neuronal activity in the brain and is associated with alterations in electrographic pattern, consciousness, sensation and behaviour [2, 3]. Secondary epilepsy may result from various insults like anoxia, trauma, tumours, metabolic abnormalities, neurotoxicity, drug withdrawal and encephalitis [3].

Epileptogenesis is a slow process. After several months of initial insult, spontaneous recurrent seizures begin to appear [3]. Epilepsy is considered to be resolved for individuals who are seizure-free for the last 10 years, with no seizure medicines for the last 5 years [1].

Although lots of antiepileptic drugs are available in the market, almost 33% patients continue to present seizure [4].There comes the importance of animal models, especially newer models which can model epilepsy in a more realistic way. However, there is a growing concern that conventional tests (MES and Sc. PTZ) identify only those drugs which have similar characteristics with existing drugs and these methods may fail to identify agents acting by novel mechanisms [5].

To understand epilepsy better, we need to understand animal models better and vice-versa. Lots of new animal models are coming up. Models are even designed for epileptogenesis and pharmacoresistant epilepsy. Lots of research activity is required in these areas to bring out novel drugs which can treat, cure and prevent epilepsy better [4-7].

Although there are lots of review articles on disease models of epilepsy, maximum are reviews on only few models and the topic seems to be partially covered. After extensive search, also we could not get a single article which encompasses every aspect of disease models of epilepsy, from simple animal models to of genetic models, models of epileptogenesis, pharmacoresistant epilepsy, computational models and alternative to animal models. Hence this work was undertaken.

Models of generalised seizures

There are lots of models for generalised seizures. Few are discussed here.

Maximal electroshock seizure

This is a model of acute seizure and one of the gold standards in early stage of antiepileptic drug screening and probably the best validated preclinical test to predict effectiveness of drugs against Grand mal seizure [4,5]. The pattern of electrographic and behavioural patterns of these seizures is similar to human seizure [8]. This model identifies those compounds which prevent seizure spread [9].

Seizure can be induced with the help of either trans-corneal or trans-auricular electrodes. But there are lots of differences between seizures induced by these two methods as they tend to activate different parts of brain. Corneal electrodes preferentially activate forebrain structures.

Tran-auricular electrodes activate brain stem and hence more effective in eliciting tonic convulsions. Seizure occurring during trans-auricular electrode stimulation occurs lower seizure threshold and is difficult to inhibit with anticonvulsant drugs than seizures induced by corneal electrodes. Again drugs preventing seizure spread are more effective in MES induced by corneal electrodes [4].

In the MES test, an electrical stimulus of 0.2 s in duration (50 mA in mice and 150 mA in rat at 60Hz) is delivered via corneal electrodes primed with an electrolyte solution containing an anaesthetic agent [9].

Local anaesthetic ensures adequate anaesthesia, contact and decreases an incidence of fatalities in mice [4].

After application of stimulus, tonic seizure occurs with extension of fore and hind limbs and body becomes stiff. At the end of tonic phase clonic seizures start, after 20-30 seconds generally the animal can come back to original position [4]. An agent which decrease the duration [10, 11] or abolish hind limb tonic extensor component of MES indicates it has ability to inhibit seizure spread [8,9].

In supramaximal current, MES test restricts itself to Na^+channel blockers. But another variant of this test, the threshold MES test, it is possible to detect the effect of the drugs even on seizure threshold. With determination of individual seizure thresholds, it extends susceptible other categories antiepileptic drug e. g. GABA enhancing drugs, glutamate receptor antagonists etc. [4].

Subcutaneous pentylene tetrazole (PTZ) method

This model identifies compounds that raise seizure threshold [9]. Drugs effective against this seizure model are potential therapies for non-convulsive seizures (absence seizure) [9,12]. The subcutaneous convulsive dose of PTZ (produces clonic seizure in 97% animals lasting for at least 5 second duration i. e. CD97) for mouse is 85 mg/kg and in rat 70 mg/kg. Animals are observed for 30 minutes [9].

The animal shows altered behavioural responses like vibrissae twitching, myoclonic jerk may be with associated vocalisation and straub's tail, loss of righting reflex but regaining the same after few seconds, freezing movements, breathing is increased, jumping and proceed to clonic seizure and lastly hind limb tonic extensor phase [12]. Absence of clonic phase in the observed period indicates that the compound under investigation increases seizure threshold [9].

MES and Sc. PTZ model are use in the primary screen while evaluating antiepileptic drugs as per NIH anticonvulsant drug development programme.

Timed PTZ infusion test

PTZ dissolved in heparinised saline 0.9% to prepare 10 mg/ml concentration. PTZ is infused at a rate of 0.5 ml per minute. Parameters observed are latency to first clonus lasting longer than 5 sec and appearance of tonic extensor phase of forelimb and/ or hind limb (TE). Infusion is stopped once TE is seen. Threshold doses for appearance of tonic and clonic seizure is calculated by the formulae

$$\frac{\text{Rate of infusion (ml/min)} \times \text{Concentration of PTZ (mg/ml)} \times 1000}{60 \times \text{body weight of the animal (gram)}}$$

This test is claimed to show more consistent result as variability of absorption don't come into account. Again another important advantage of this model is that with the help of it, we can measure seizure threshold. Sequence of response seen in this test are vibrissae twitching, myoclonic jerk with vocalisation, clonus and forelimb/hind limb tonic extensor [12].

Homocysteine

When rats or mice are given homocystein thiolactone 1 g/kg i. p. they develop Generalised tonic clonic seizure with a latency period of almost one hour [13].

Models of generalised seizure (absence seizure)

Although lots of animal models are available for absence seizure including genetic models, only few are discussed below.

WAG/Rij model

It is a strain of inbreed rats which show spontaneous spike and wave pattern in EEG with associated behavioural abnormalities as seen during human absence seizure. Drugs active against absence seizure are supposed to decrease these electrographic and behavioural abnormalities [14-16].

Gama hydroxybutyrate model (GHB)

Administration of GHB causes changes of EEG and behavioural patterns which mimic human absence seizure. Rats when injected with GHB 100-150 mg/kg the shows periodic bursts of 4-6 Hz spike wave activity [13].

GAERS genetic absence epilepsy rat from strasbourg (GAERS)

These animals show recurrent generalized non-convulsive seizures with behavioural arrest, staring and vibrissae twitching, which is accompanied by bilateral and synchronous spike-and-wave discharges (SWD).

ntiabsence drugs are effective (e. g. valproate, ethosuccimide) but drugs specific for convulsive or focal seizures (phenytoin, Carbamazepine) are ineffective in suppressing the SWDs [17].

Penicillin model of absence seizure

Penicillin G when given by intramuscular route (≥3 lac unit /kg) to cat, epileptiform activity begins after 1 hour which is characterised by repeated arrested activity, myoclonus, staring and occasionally progressing to GTCS. This model shows spike wave discharge with normal background activity in EEG as seen in human absence seizure [13].

Another method of inducing absence seizure is subcutaneous injection of pentylenetetrazole (85 mg/kg). EEG of the treated animals shows spike wave discharges. Drugs effective in preventing PTZ induced seizure are effective in managing human absence seizure [9].

Models of infantile spasm

Infantile spasms are a type of severe developmental epilepsy which begins in the first year of life, commonly between 3 and 10 months of age. The neural circuits become epileptogenic after weeks or months of an initiating injury. Spasms may respond to glucocorticoids, ACTH (adrenocorticotropic hormone) and vigabatrin. Unfortunately most anticonvulsants are ineffective in this disorder [18-20].

Corticotropin-releasing hormone (CRH) model

Intraperitoneal or intracerebroventricular administration of CRH, during the second week of life in rats, causes severe seizures [18, 21]. Much higher doses of exogenous CRH are required to produce seizures at older ages [19].

Betamethasone-NMDA model

Here glucocorticoid (betamethasone administered to pregnant rats on gestational day 15) serves as a prenatal stressor which predisposes the exposed immature foetal brain to infantile spasms. Glucocorticoid sensitizes the foetal brain to postnatal NMDA. The same pups are given NMDA on postnatal day15 (P15). They shows several changes like tail twisting and flexor spasms, which are sensitive to ACTH. This model is said to be a model of cryptogenic infantile spasms [17, 21].

Tetrodotoxin (TTX) model

Intrahippocampal infusion of the sodium channel blocker, tetrodotoxin (TTX), beginning on Postnatal day 10 to 12 (with the help of chronically implanted with osmotic minipumps in the neocortex or hippocampus) caused recurrent brief spasm like seizures in rat pups [18, 22]. Spasms occur singly or in clusters. The interictal EEG pattern is similar to hypsarrhythmia found in infantile spasm [19].

Multiple-hit model

In multiple hit model, severe damage is caused to cortical and subcortical structures. On postnatal day 3, lipopolysaccharide is injected intracerebrally and doxorubicin is administered into the cerebral ventricles. These compounds cause cortical and sub-cortical injuries.

Serotonin decreases brain excitability. To overcome this problem, on postnatal day 5, p-chlorophenylalanine (blocker of Tryptophan hydroxylase) is injected intra-peritoneally to deplete brain serotonin by blocking its synthesis. Treated animals develop recurrent seizures from postnatal day 7 to 11, which consisted of spasms in clusters. After postnatal day 11 a transition is seen and seizures change into limbic seizures. This mimics transition from infantile spasms to other seizure types that occurs in human [18, 19].

ARX (Aristaless-Related Homeobox) mutation model

The ARX gene encodes transcription factors that are necessary for brain development. In mice models of ARX mutation, variety of neurologic defects are seen including mental retardation, epilepsy and including infantile spasms [18,19].

Down syndrome model- Ts65Dn mouse

10% of children with Down syndrome show evidence of infantile spasm. Ts65Dn is mouse model of Down syndrome. A normal 1 week to 2 months old Ts65Dn mice normally don't show any clinical

evidence of seizure. But when they are exposed to baclofen or γ-butyrolactone (prodrug of γ-hydroxybutyrate), the animals develop clusters of extensor spasms accompanied by similar EEG changes as occurs in human infantile spasm. More importantly, ACTH, valproic acid, and vigabatrin are succesful in preventing spasms in this model [19].

Model of myoclonus

Post hypoxic myoclonus in rats

In anaesthetised rat, left femoral artery is cannulated to monitor blood pressure and left femoral vein administration of epinephrine for resuscitation. Cerebral hypoxia was induced by mechanical compression of the aorta and major cardiac vessels by a "L" shaped loop such that arterial blood pressure reduces to 0–5 mmHg. After 8 minutes and 30 seconds, resuscitation should be done with 10 µg/kg epinephrine and 4 mEq/kg sodium bicarbonate (intravenous injection) and manual thoracic compressions should be given until the systemic blood pressure of the animal was returns to normal preprocedure level. The animals show spontaneous seizures and audiogenic myoclonic jerks [23]. Hypoxia can also be induced by cardiac arrest (By intracardiac injection of potassium chloride) [15].

Models for partial seizures

Bicuculine

When bicuculine is topical applied on sensory motor cortex, bicuculine treated mice serve as a model of acute simple focal epilepsy [13].

Bicuculine as a model of chronic simple partial seizure (model of systemic focal epileptogenesis)

Limited volume of rat cerebrum is irradiated to disrupt blood brain barrier. 3-6 months later bicuculine methionide (2mg/kg) is to be injected systemically. Epileptic focus will be induced which will show EEG spikes and features of focal seizure [13].

Penicillin model of acute focal seizure

When Penicillin applied topically to rat cerebral cortex (1.7-3.4 mM concentration) with the help of cottonoid pledget, animals start showing features suggestive of acute focal seizure. This is a popular model to study simple partial seizure [13].

Alumina hydroxide

This is a model of spontaneous recurrent simple focal epilepsy. 4% aluminium hydroxide is injected into surgically exposed sensorimotor cortex. Recurrent seizures begin after 1-2 months and continue for years [12, 24].

Zinc sulphate

This is a model of complex partial seizures with secondary generalisation when zinc sulphate is injected Intra-hippocampally [10 µl of zinc ($ZnSO_4.7H_2O$, 200µg/kg)], animals show evidence of complex partial seizure which may secondarily generalise [13, 24].

Colchicine

Rats and cats develop focal epilepsy when colchicine in agar is applied focally to neocortex [13].

Kindling

The behavioural and electrographic components of kindling have similarity with human partial onset seizures as it may undergo secondary generalisation. Kindling may be electrical or chemical.

Electrical kindling

Electrical kindling can be produced by stimulation of specific areas of brain (most commonly hippocampus and amygdala). Also corneal electroshock kindling also can be done in animal e. g. in mice and rats. Mice are kindled by ones daily stimulation of 3mA for 2 seconds daily while rats are kindled by stimulating twice daily with 8mA for 4 seconds (until racine stage 5 seizure is observed). Stimulation of the hippocampus and cortex also produces kindled seizures [6, 9].

Chemical kindling

The repeated administration of sub-convulsant doses of PTZ causes progressive sensitization to its convulsant effects. Kindling can be observed using two different treatment schedules: 1) PTZ 30 mg/kg, IP daily or 2) 30 mg/kg, IP every alternate day for eight weeks. With these regimes, more than 80% of the animals show convulsions by the end of treatment[25].

RRHS model

In rapidly recurring hippocampal sclerosis model, hippocampus of rat is stimulated with 10 second trains of titanic electrical stimuli (suprathreshold) every few minutes. Parameters observed are behavioural seizures, electrographic alterations, threshold of afterdischarge and duration of afterdischarge [6].

Kainic acid model of temporal lobe epilepsy

Kainic acid (KA) is an analogue of glutamate. It is a potent agonist of glutamate receptors (AMPA/kainate class). Systemic and intracerebral administration of KA (4 mg/kg i. v., or 0.8-2.0 µg intra-hippocampally) initially induces seizure which last for hours, followed by a latent seizure-free period of 3-4 weeks after which recurrent spontaneous focal seizures starts appearing. that begin between 3 and 4 weeks. KA has special affinity to hippocampus. Even when given either systemically or at sites far away from hippocampus, KA shows prominent effect on hippocampus. KA model can be used as a model of human temporal lobe epilepsy (many features are common to both such hippocampal sclerosis and mossy fibre sprouting) and epileptogenesis (excitotoxic neuronal loss occurs in the dentate gyrus and CA1 and CA3 region of hippocampal subfields and subsequent axonal reorganisation as occurs in human epilepsy. There is a direct correlation between damage of hippocampal subfields and epileptiform activity). Similar KA model is reported in mice also. Mice treated with KA shows similar epileptic behaviour and histological evidence of acute neuronal loss in the CA1 and CA3 regions of hippocampus [24, 26].

Tetanus toxin

This is a model of recurrent, chronic partial seizures. A single unilateral injection into the hippocampus produces neuronal loss in the CA1 pyramidal cell layer of hippocampus [24].

Cobalt: Microinjection of cobalt chloride into lateral cerebral ventricle produces seizure in rats which is clinically similar to kainite induced seizures and amygdale kindling [13].

Models of pharmacoresistant seizures

Despite the use of optimal use of currently available anti-epileptics, almost one third of patients continue to suffer from seizure [4, 9].

Although maximal electroshock and pentylenetetrazole are fruitful models, they are reported to identify drugs acting by same type of mechanism of action [5, 27].

For better understanding, targeting and discovery of compounds for pharmacoresistant epilepsy, the need of the day is an appropriate animal model [27].

***In vivo* models**

6-Hz "Psychomotor" Seizure Test

MES and s. c. Met tests although extremely effective in identifying new drugs that may be useful for the treatment of generalized tonic-clonic and myoclonic seizures, [28] they may miss novel antiepileptic drugs that could be useful for the treatment of therapy-resistant partial seizures. Hence potential antiepileptic drugs are also screened for their ability to block seizures in the 6 Hz psychomotor seizure test. Here psychomotor seizures are induced by a low-frequency (6 Hz) but long-duration (3 sec) stimulus delivered through corneal electrodes. Initially 32 mA intensity is used and observed for the presence or absence of seizure activity but later stimulation current may be increased to 44mA. For example, levetiracetam is highly effective in the 6 Hz model but it is

ineffective in the MES test. This type of psychomotor seizure is characterized by minimal clonic phase which is followed by stereotyped, automatistic behaviours (e. g. twitching of vibrissae) and Straub-tail. After treatment by an agent, if the animals do not display such behaviours, they are considered protected. Seizure score can be used as additional measure. These behaviours seems similar to the aura of human patients with partial seizures [9].

Corneal kindled mouse

This is a model of human partial epilepsy with secondary generalisation. Electric stimulus is applied trans-corneally to the brain through optic nerve. After applying 0.5% tetracaine hydrochloride solution to each eye, corneal electrodes are applied and a current of 3 mA, 60 Hz for 3 seconds is applied. This procedure is to be repeated twice daily. Stimulations continue till the mouse is fully kindled (5 consecutive stage 5 seizures). Agents are tested 7 days after last stimulation. A seizure score of < 3 is considered protective [9].

The behavioural seizure scores are rated as: Stage I - clonus in mouth and face, Stage II – stage 1 with associated head nodding, Stage III - forelimb clonus, Stage IV - stage 3 with rearing, Stage V - stage 4 with repeated rearing and falling [29].

Hippocampal kindled rat model

Hippocampal kindled rat is an experimental model of focal seizures that become secondarily generalized. This model is useful for identifying compounds effective against partial seizures, compounds affecting seizure spread and generalization. Adult, Sprague-Dawley rats (male) are taken and bipolar electrode is stereotaxically implanted (Anaesthesia used is ketamine-xylene) into the ventral hippocampus. After that rats are allowed to recover for one week. For kinding 50 Hz, 10 sec train of 1 ms biphasic 200 μA pulses to be given every 30 min for 6 hours, for five days on every alternate day. Fully kindled animals start showing consistent Racine stage V behavioural seizure. Agents are evaluated after one week of stimulus free period [9].

An effective compound lowers behavioural scores and duration of afterdischarge compared to control. Suppressing or delaying acquisition of the kindled response indicates that the compound under investigation can have antiepileptogenic action [9].

Lamotrigine resistant amygdale kindled rats

In amygdala kindled rats, exposure to low doses of lamotrigine during kindling development leads to resistance development to the drug in the subsequently kindled animals. Lamotrigine resistant kindled rats are refractory to phenytoin and carbamazepine. This model is proposed to be a model of drug resistant epilepsy [5].

Methylazoxymethanol acetate induced cortical dysplasia

Pregnant rats are exposed in utero to MAM. These neonates show some cortical dysplasia like syndrome in their adulthood. Seizure induced in these rats by kainite is refractory to many antiepileptic drugs including Carbamazepine and valproate [5].

There are lots of other models are there such as Phenytoin resistant amygdala kindled rats, [5]. benzodiazepine-resistant convulsive and non-convulsive status epilepticus test etc [9].

***In vitro* models**

Organotypic hippocampal slice culture model of pharmacoresistant epilepsy

In organotypic hippocampal culture, cell types and most of their connections are preserved. If such cultures are exposed to low Mg^{2+}or 4-AP, they show robust evidence of status like activity. Diazepam fails to control this high epileptiform activity even in very high doses. However anaesthaetic concentration of phenobarbitol could control these status like events. [7,9,30].

Models for status epilepticus

Generally status epilepticus can be induced by electrical or chemical methods but it can also be induced *In vitro* in organotypic hippocampal slice culture models.

In vivo

Repetitive stimulation of hippocampal afferents (e. g. perforant path), amygdala and hippocampus are known to produce status epilepticus. Again status epilepticus can be induced by chemical method also. Many agents like pilocarpine, lithium-pilocarpine, diisopropylflurophosphate, kainic acid are widely used to evaluate and characterise antiepileptic drugs [3].

Kainic acid model

ICV (intracerebroventricular) injection of 0.4-0.8 μg kainic acid or systemic injection of I. P. or s. c. injection of 8-12 mg/kg kainic acid induces chemoconvulsion which progresses to develop into status epilepticus [3, 31].

Pilocarpine model

Pilocarpine when administered systematically in high doses to rats (400mg/kg) or mice (300-350 mg/kg) the animals first develop limbic seizures which progress over time to limbic status epilepticus [3, 32, 33].

Lithium- pilocarpine model

To enhance the consistency of pilocarpine, small amount of Lithium (LiCl 3mEq/kg (i. p.) is given to the animals 24 hours prior to administration of pilocarpine (20 mg/kg i. p.). 1mg/kg s. c. [3, 34, 35]. Scopolamine is to be given 30 minutes prior to pilocarpine administration to counteract its peripheral effects. Treated animals show behavioural and electrographic evidence of status epilepticus for over 5 hours [3].

There are numerous other chemical models of SE like Flurothyl model, Cobalt-Homocysteine model etc [3].

In-vitro

Low or zero Mg^{2+}model in entorrhinal- hippocampal slices

The zero Mg^{2+}model in entorrhinal-hippocampal slices is of particular importance. [36-39]. Zero level of Mg^{2+} produces synchronised epileptiform discharges in hippocampal slices, the first stage is epileptiform discharges that are sensitive to benzodiazepine but in the second stage (occurs after around one hour), late recurrent discharges occurs which are insensitive to benzodiazepines [36, 38].

4-aminopyridine is a popular in-vitro method because of it is a potent epileptic agent and the actions are highly reproducible in slices. Again another variety called organotypic hippocampal slices are also commonly used to study status epilepticus and to evaluate drugs effective against it [3].

Genetic models of epilepsy

There are many seizure prone animals; epilepsy in these animals closely mimics human epilepsy. Some important and validated models are discussed below.

Spontaneous epileptic rats

when tremor homozygous rats are allowed to mate with zitter homozygous rat, the new progenies become spontaneously epileptic. They show behavioural responses like wild jumping and tonic convulsions in absence of any external stimuli [6, 15].

Audiogenic seizure prone mice (DBA/2J)

On exposure to high frequency high intensity sound (10-20 kHz, 90-120 dB) they show behavioural alterations like wild running and generalised tonic clonic seizure [6, 15].

Genetically epilepsy prone rats (GEPR rats)

It is one of the best known genetic epilepsy model. Both GPER 3 and GPER 9 has moderate to severe degree of seizure predisposition. GPER 3 responds to a sound stimulus by generalised clonus and GPER 9 presents with tonic extensor convulsion. In GPER, input from

forebrain circuit activates brainsten seizure circuit which results in complex partial seizures with secondary generalisation [6, 40].

Tottering mouse (tg/tg strain)

Shows 6-7 per second spike wave discharges. Anti absence drugs are effective in this model [6, 15].

Quacking mouse

This type of mouse when handled by tail and slowly rotated, exhibits generalised tonic clonic seizures [15].

EL mouse

It is a model of hereditary focal epilepsy precipitated by sensory stimulation (vestibular stimuli such as spinning or tossing, animals show feature of complex partial seizure) [15, 24]**,** Other important genetic models of epilepsy are Photosensitive fowl, [6,15]. Mongolian gerbils (Meriones Unguiculatus), [6]. Photosensitive baboons (Papio Papio), [6, 15]. Epileptic dogs, Mocha, Stargazer and Lurcher [6, 15].

Alternative to animal models in epilepsy

The zebra fish model (Danio Rerio):

Wild type larval zebrafish is to be taken. PTZ is dissolved in the bathing medium at a concentration between 2.5 to 15 mM at pH 7. Freely swimming zebra fish are exposed to PTZ in a petridish or 96 well felcon plates. Electrophysiologic studies can be done by embedding the fish on agar plate.

Its behaviour after exposure to PTZ is graded as stage-1, increase in swim activity, stage 2, rapid circling along the outer edge of the well, stage 3, brief head to tail convulsions followed by free floating (loss of posture) [41, 42].

Other important models of epilepsy are Caenorhabditis elegans (worms) and Drosophila melanogaster (Fruit flies) [41].

The *in vitro* mechanism of action test

In an effort to gain insight into the potential mechanism of action of the anticonvulsant compound under investigation, its interactions with voltage- and receptor-gated ion channels are examined using whole-cell patch-clamp electrophysiology techniques in cell cultures. In whole-cell patch clamp recording technique, cortical cells from 15-gestational-day-old Swiss Webster mouse fetuses are cultured and are used 2-3 weeks after plating. Whole-cell recordings from a murine neuroblastoma (N1E 115) cell line are used to characterize the effects of the test compound on voltage-gated sodium channels [9].

Other effects observed are voltage-activated Na currents in cultured neocortical neurons, effect of the drug on GABA-induced Cl– fluxes across the membrane in mouse cortical neurons and cerebellar granule cells, effect of the drug on kainite and NMDA evoke inward current on cultured hippocampal neurons etc. Receptor binding and neurotransmitter uptake studies can be performed on GABA, NMDA and monoamine receptors, or on GABA, adenosine, dopamine, serotonin, norepinephrine and glutamate transport systems [43].

Models targeting epileptogenesis

Current used models e. g. the MES model and the PTZ model are models of seizures (ictogenesis), but they are not models of epileptogenesis [27]. Hence, there is a need for properly validated models of epileptogenesis which can be used for screening of newer chemical entities [4].

No current antiepileptic drug can prevent or modify epilepsy occurring after brain insults (post-traumatic epilepsy) [44]. The proposed models are post status epileptius- temporal lobe epilepsy (Post SE-TLE), Models of kindling and traumatic brain injury (TBI) [4].

In Post SE-TLE model, first SE is induced by electrical or chemical stimulation (Pilocarpine or Kainate). After 1-4 weeks post SE, the animals starts showing spontaneous or recurrent seizures with or without hippocampal damage. The time from initial insult to development of epilepsy is the period of epileptogenesis. Enormous effort is going on to develop new antiepileptic drugs [4].

Computational models

The computational models can be classified as Deterministic micro-scale model, Detreministic macro scale model, Non-deterministic stochastic models, and Non deterministic statistical models. Micro scale models are concerned with dynamical behaviour of neurons, neuronal morphology and ion channels. Neural networks are constructed using Hodgkin Huxley framework. Macro-scale models try to explore dynamics of neuronal population rather than concentrating on individual neurons.

They try to explore dynamics resulting from interaction of many brain areas or regions. Non deterministic models focus on inherent variability lying in the recorded brain activity. There are lots of non deterministic statistical models are there like autoregressive model, generalised linear models, structural equation models.

To enhance efficacy of these tools, these are supported by advanced techniques like support vector machines and artificial neural networks. Hidden marcov model and marcov chain model has significant application in epilepsy research [45].

Other important computational models used in epilepsy research are realistic models of the corticothalamic loop, Compartmental models of hippocampus, time independent models of seizure intervals, time dependent models of seizure intervals etc [46].

Databases of published models can be searched at ModelDB. Some related databases are CoCoDat, The Database of Cortical Microcircuitry". To run simulations some tools are there like MATLAB, Mathematica, PyDSTool etc. Softwares used solely for neuronal modelling are NEURON, Brian, Nest, Parallel neural Circuit Simulator and Genesis [46].

CONCLUSION

In summary, the above discussed models can be used to identify and characterise new chemical entities for treatment of epilepsy. Primary screening by NIH primarily concentrates on MES and Sc. PTZ test. Characterisation of anticonvulsant compound is not the final job. These compounds are also to be tested for neurotoxicity (Rota rod test, gait and stance test), Proconvulsant evaluation (intravenous PTZ threshold test) and tolerance and metabolism studies along with safety issues before entering into next phase [9].

Animal models are playing the main role in anticonvulsant screening and it is unlikely that they may be replaced by in-vitro methods although they can supplement them [4]. Pharmaceutical industry primarily uses mechanism specific approach as primary screening tool and mechanism independent models are used to verify different mechanism based hypothesis. Seizure type models are used for secondary evaluation [6].

Different models of epilepsy significantly improved our understandings about epieptogenesis and ictogenesis [5]. We need new and more validated animal models which can mimic different types of human epilepsy more closely and improve our understandings about epilepsy far better.

CONFLICT OF INTEREST

Nil

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