

Pentilenetrazole Kindling Epilepsy Model

Pentilentetrazol Tutuşma Epilepsi Modeli

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Summary

Epilepsy is one of the most common neurologic disorders affecting approximately 1% of the general population, and no alleviation was achieved in one third of the patients medicated with antiepileptic drugs. Current treatments of epilepsy are symptomatic and have more anti-seizure effects than antiepileptic effects. These therapies do not cure epilepsy. Basic mechanisms of epilepsy have not entirely been clarified yet. Using seizure and epilepsy animal models, our comprehension about basic mechanisms underlying epileptogenesis has improved. In addition, animal models of epilepsy and seizures are very useful in the discovery and development of new antiepileptic drugs. Pentilenetrazole (PTZ) is widely used in antiepileptic drug discovery studies, and PTZ kindling model is very important to understand the pathophysiology of epilepsy. In this review, current information about PTZ kindling model was given in this aspect.

Key words: Chemical kindling; epilepsy; experimental animal models; pentilenetrazole; rat.

Özet

Epilepsi genel popülasyonun yaklaşık %1'ini etkileyen en yaygın nörolojik düzensizliklerden biridir. Antiepileptik ilaç tedavisi gören hastaların üçte birinde hiçbir hafifleme elde edilememektedir. Epilepsi için kullanılan güncel tedaviler semptomatiktir ve antiepileptik etkiden ziyade anti nöbet etkilidir. Bu tedaviler epilepsiyi tedavi etmemektedir. Epilepsinin temel mekanizmaları da henüz tam olarak anlaşılamamıştır. Nöbet ve epilepsi hayvan modelleri kullanılarak epilepsinin temelinde yatan mekanizmaları kavrayışımız ilerlemektedir. Ayrıca epilepsi ve nöbet hayvan modelleri yeni antiepileptik ilaç keşfi ve gelişimi bakımından kullanışlıdır. Pentilentetrazol (PTZ), antiepileptik ilaç keşif çalışmalarında yaygın biçimde kullanılmaktadır ve PTZ tutuşma modeli epilepsinin patofizyolojisinin anlaşılması bakımından oldukça önemlidir. Bu derlemede, PTZ kindling modeli hakkında güncel bilgiler sunulmaktadır.

Anahtar sözcükler: kimyasal kindling; epilepsi; deneysel hayvan modelleri; sıçan; pentilentetrazol.

Introduction

Epilepsy is a brain disorder including unpredictable and repetitive seizures that interrupt normal brain functioning.^[1] Epilepsy is one of the most common neurological clinical-pathological disorders that affect about 1% of the general population.^[2]

Seizure activity can be controlled with current therapies however they don't prevent or cure epilepsy. Anti-seizure drugs have low neuroprotective activity or their side effects which are the outcomes of long therapy overcome their therapeutic benefits. Therefore considerations now

focus on neuroprotective effects of various components.^[3]

Considerable numbers of clinical and epidemiological studies reveal that about one third of adult patients (20-30%) are suffering from epilepsy and they don't respond to drug therapy or surgical treatment^[4] and they face with resistant type epilepsy.

Experimental animal models are used in order to explore basic mechanisms underlying epilepsy and to discover new antiepileptic drugs (AEDs), since intracellular recording of intact human brain, microchemical analysis and anatomic investigations are not possible because of ethical reasons.^[5]

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Epilepsy

World health organization states epilepsy among the most common and serious brain disorders.^[6]

According to etiological classification epilepsy can be ordered in 4 groups such as idiopathic, cryptogenic, provoked and symptomatic.

1. In idiopathic epilepsy generally a genetic cause is found and no neuropathological or neuroanatomical anomaly exists. It is defined as an epilepsy type occurred by multi genetic or by complex inheritance factors.
2. Cryptogenic epilepsy is defined as an epilepsy type which has a symptomatic nature without a known cause.
3. In the provoked type again no given important neuro-anatomical or neuropathological anomaly is found. Seizures are mostly formed by specific environmental or systemic factors. In some of the provoked epilepsy cases a genetic makeup is found as the underlying mechanism. However, no inherited reason was found in most of the cases.
4. Symptomatic epilepsies may have causes such as trauma, tumor, infection, malformation or a systemic genetic disease. In symptomatic epilepsy an inherited or a genetic cause is present. Serious anatomical or pathological anomalies and/or clinical findings are indicators for underlying disease.^[7]

Experimental epilepsy models

Experimental epilepsy studies are performed for three reasons; drug discovery, mechanism clarification and identification of interrelations between major events and processes.^[5]

Animal models constituted for epilepsy and seizure are of vital importance for augmenting our understanding for the basic mechanisms underlying epileptogenesis, discovery of new AEDs, determination of clinical effects of new AEDs, approval of those drugs for safety on human health.^[8,9] and for designing specific models for drug resistant seizures (Table 1).^[9]

Electrical or chemical kindling are epileptogenic models used for understanding the epileptogenic process and for studying molecules that are preventing this process.^[10]

Table 1. Frequently used tests for new AEDs

I. Acute models
i. Maximal electroshock seizure (MES)
ii. Pentylentetrazol (PTZ) induce seizure
II. Chronic models
i. MES (electrical) kindling
ii. PTZ (chemical) kindling (chronic models). ^[8]

Kindling model

Kindling is a phenomenon resulted with progressive intensity of convulsion activity due to repetitive administration of electrical or chemical sub-convulsive stimulators.^[11] If the stimulus causes generalized convulsion in experimental animal it is accepted that kindling is completed and it is agreed that this abnormal excitable status remain permanent.^[12]

Pentylentetrazole (PTZ) administration is a commonly preferred behavioral approach used for studying brain excitability^[13] and for developing AEDs.^[14] Chemical kindling seizures induced with PTZ are human absence epilepsy and myoclonic, generalized tonic-clonic (primary generalized) seizure models^[15] and it is a model for drug resistant epilepsy.^[16] Kindling became one of the most important epilepsy model used for investigating neurochemical and long term structural changes in the brain.^[17] Since it was invented by Goddard at 1967,^[18] kindling has been used as a chronic animal model for temporal lobe epilepsy (TLE)^[19] and also as a very important model for complex partial epilepsy.^[5] Goddard implanted some bipolar electrodes to different sub-cortical regions of the brain in his studies on rats. He also stimulated those regions with various current strengths. Goddard obtained no behavioral or electrographical response to any sub-convulsive stimulus at the beginning. However he observed that when repetitive administrations of those sub-convulsive doses are applied to rats, finally fully achieved convulsions occur following some certain stimulus. He also found that, because of the permanent alterations in the brain obtained with kindling, kindling can be achieved as a response to a new sub-convulsive stimulus in kindled animals. Goddard produced the term "kindling" for such progressive changes in the brain.^[20]

Due to firing in PTZ kindling model following events occur;

- Alterations in emotional behavior
- Cognitive inconsistencies
- Neuronal loss in hippocampus.^[21]

5. PTZ kindling protocol

PTZ can be used for initiating acute (60 to 100 mg/kg) as well as chronic (sub-convulsive doses) animal models of epilepsy.^[20]

Required materials

PTZ (Sigma, Aldrich), physiological saline (for dissolving PTZ), chronometer (for determining latent period and seizure time), video camera (in order to record seizure activity of animals for 30 minutes after injection) are used.

Number of animals

For PTZ kindling (chronic model) a rodent such as rat or mice can be chosen.

Generally, it is advised to keep number of individuals at 10 at the beginning of the study. Because some animals may die before obtaining required data. A minimum of 6-8 animals should be alive when the protocol is finished for achieving the data to be statistically meaningful.^[20]

Experimental protocols

Two types of experimental protocols exist for assessment of drugs in kindling: 1) drug is administered before each stimulus and its effect on acquisition of kindling is determined by comparison with control group; 2) Effects of anticonvulsant drugs are evaluated in fully kindled rats.^[22]

Standard PTZ kindling model needs electrode implants when behavioral and electrophysiological experiments will be performed. In contrast it doesn't need electrode implants when the researchers focus on behavioral and molecular experiments.^[23]

PTZ can be used for developing acute as well as chronic (kindling) animal epilepsy models. For example, acute injection of PTZ at threshold dose (60 to 100 mg/kg, i.p. or s.c.) to rodents, myoclonic jerks, clonus, tonic extensions occur. However, repetitive administration of PTZ at sub-threshold doses (20 to 40 mg/kg, i.p.) produces kindling phenomenon.^[20]

Protocol for producing kindling in mice according to literature is as follows: 1, 3, 5, 8, 10, 12, 15, 17, 19, 22 and 24th days of the study (total of 11 injections in days of Monday, Wednesday and Friday) PTZ is injected at sub-convulsive

doses (35 mg/kg i.p.) and PTZ-kindling model is formed. A single 75 mg/kg challenge dose is administered on day 26.^[24]

Kindling protocol in rat is given as follows in literature records:

1. Regular administration of PTZ at sub-convulsive doses (30 mg/kg i.p., 3 times a week, up to 10 weeks), generate chemical kindling in 80% of the rats.^[25]
2. Protocol given in male Wistar rats are as follows: intraperitoneal 35 mg/kg PTZ injection is administered at every 48 hours (Monday, Wednesday and Friday of the week). Following 20 consecutive injections treatment is ceased for 9 days. Then, animals are tested with 21st to 25th injections of PTZ and protocol is completed.^[26]
3. A novel protocol is developed and presented by Davoudi et al., on rats to constitute PTZ kindling model.^[23] It is named as Win-PTZ kindling method. In this method 4 doses of PTZ injections are administered into rats at the beginning. No injections were administered for the following 22 days. Then last 3 PTZ injections are administered at the 29th, 31st and 33rd days. At the end of those 3 injections complete kindling criteria are established. Number of animals within each group is set to 8 in this model.

PTZ kindling protocol can be generated with one of the applications listed above. Seizure scores 30 minutes after each PTZ injections are defined as follows:

- Phase 0: No response
- Phase 1: Ear and facial twitching
- Phase 2: myoclonic body jerks
- Phase 3: clonic forelimb convulsions
- Phase 4: generalized clonic convulsions, turning onto one side position
- Phase 5: generalized clonic-tonic convulsions (or death within 30 minutes).^[27]

(Some of the researchers in the literature consider mortal seizures occurring after tonic clonic seizure phase 5 under an additional 6th phase category).

- Phase 6: Mortality.^[28]

Again, according to Dhir, seizure scores are evaluated under following headings after PTZ injections:

- No seizure behavior, (is calculated as score 0)
- Myoclonic jerks, (is calculated as score 1)
- Straub's tail, (is calculated as score 3= 1 score for myoclonic jerk+ 2 score for Straub's tail)
- Clonus, (is calculated as score 6= 1 score for myoclonic jerk+ 2 score for Straub's tail+3 score for clonus).^[20]

Racine's scale was originally designed for amygdala-kindling model and prepared by comparison of electroencephalographic (EEG) records in seizures. In this aspect, use of the same scale for PTZ kindling is criticised by some authors. For that reason Racine's scale was revised for PTZ kindling model by Lüttjohann et al.^[29] Generally different brain regions are evaluated for epileptic changes following kindling by PTZ protocol due to their different physiological functions and according to their pathophysiological states^[30] (e.g. cortex, cerebellum, pons-medullar regions^[30] and basal ganglia (nucleus caudatus, putamen).^[31] However in all of the studies hippocampus region is considered commonly.

EEG is used in electrophysiological studies for confirmation of observed seizure intensity in behavioral aspect. Surgical interventions for EEG recording are performed under anesthesia (ketamine, 100 mg/kg, ip and xylazine 20 mg/kg ip).^[32] Animals are placed in the stereotaxic frame.^[33] By the guidance of stereotaxic atlas, electrodes are placed into the intended coordinates.^[29] They are fixed with screw, covered with cold acrylic and sculp is sutured.^[32] Following the surgery, 1 week is passed for healing of local effects of surgery and anesthesia before monitoring procedure.^[33]

Action mechanism of PTZ

PTZ is a selective antagonist of receptor of GABA_A chloride ionophore complex. It has convulsive activity when used alone or in repetitive doses. It affects GABAergic and Glutamergic systems in many brain regions including hippocampus.^[34]

PTZ application causes hippocampal atrophy in rats. Selective neuronal loss and astocytosis in hippocampus are observed in PTZ administered rats. In addition, in magnetic resonance studies (MRI) on rats which are given PTZ, a decrease in cerebellum volume is visible (17). Although direct mechanism of PTZ is not known in detail, literature records reveal that it causes alterations in GABAergic systems, Glutamergic systems and antioxidant defense systems:

Alterations in GABAergic systems

Kindling produced by PTZ may be related with permanent attenuation of inhibitory function of GABAergic system in the brain.^[25] Repetitive single dose application ends up with decreased GABAergic activity.^[35]

PTZ is claimed to exert its activity via inhibiting gamma-aminobutyric acid (GABA) activated channels.^[36] It is suggested that its activity is especially due to blockade of GABA_A gated chloride receptors.^[37] GABA_A receptors have some allosterical binding sites. Different drugs can influence GABA mediated chlorine influx via those binding sites. PTZ is a central nervous system convulsant. It shows its activity by binding to site where picrotoxin (PTX) binds to GABA_A receptor and probably exerts its activity through interaction at the picrotoxin site within (TM2) GABA_A receptor subunit second transmembrane domain.^[38]

There are findings about alterations in GABA_B receptor mechanisms during kindling according to results of learning and memory studies done with PTZ kindling method.^[39] In addition, it is reported that levels of GABA transporters can be a determinant for seizure vulnerability and epileptogenesis.^[40]

Changes in glutamergic system

PTZ also causes an alteration in density and sensitivity of different glutamate receptor sub types^[41] in many parts of the brain and an increase in density of glutamate neurotransmitter at the hippocampal region.^[42] Various behavioral, neurophysiological and neurochemical changes occur during PTZ induced kindling. An increase occurs in (metabotropic) glutamate receptor density and IP₃ (Inositol triphosphate) formation during PTZ kindling.^[43] It is reported that changes in molecular expression in glutamate transporters in kindling process may trigger development of epileptogenesis.^[40] It is suggested that N-methyl-D-aspartate (NMDA) plays a role in kindling epileptogenesis. Subunit and region related alterations of NMDA receptors during synthesis in PTZ induced kindled seizure development in rats suggests that these alterations may be responsible from the spread of PTZ induced neuronal hyperactivity and seizure constitution.^[44]

Long term neocortical plasticity aroused by kindling; can be generated by an alteration in the delicate balance between neuronal inhibition and excitation (a relative decrease in

inhibition and a relative increase in excitation or a combination of both).^[39] Kindling causes an enhancement in the release of GABA in hippocampus and a decrease in GABA receptor sensitivity. PTZ induced convulsions start by glutamate receptor activation and inhibition of inhibitor GABA neurotransmitters and proceed into generalized form.^[30] As an important criteria for development of kindling, with the collapse of GABA-A-mediated inhibition, activation of NMDA receptor complex is accused.^[45]

In addition, α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) which is among the ionotropic glutamate receptors seems to play role in epileptogenetic mechanisms. In a related study it was reported that AMPA receptors play role in cortex and basal ganglia for sustaining PTZ kindling phenomenon.^[31]

Antioxidant defense systems

Oxidative stress plays a major role in many of the epilepsy models. Findings of Patsoukis et al show a strong correlation between PTZ administration and oxidative stress.^[34] PTZ kindling causes alterations in antioxidant defense systems of the brain.^[20] Oxidative stress is also present in other experimental epilepsy models as well as in PTZ kindling model. Due to oxidative stress, abnormal structural changes occur in cellular proteins, membrane lipids, DNA and RNA. Oxidative stress at brain is accepted to be the common reason for many of the acute neurological disorders including Parkinson and Alzheimer's disease which have a chronic nature.^[24]

Advantages and disadvantages of kindling

- Kindling model brings advantage for providing both epileptogenic and spontaneous seizure model.^[16]
- Time, place and period of stimulation can be adjusted. By this method potential treatments can be arranged.^[46]
- Initiation of seizure is easy with this method. Also this method's prediction for determination of clinical effects of AEDs is high.^[14]

Advantages of the Epilepsy in the MES kindling model

- It is an appropriate model for new AED discovery and improvement.^[9]
- Full activation of the targeted brain region is possible.
- Development of chronic epileptogenesis can be monitored reliably.

- Dissemination of seizure table and its evolution into generalized type can be easily observed,
- Period between seizures, ictal and postictal periods can be easily arranged.^[19]

Advantages of chemical kindling model

- Construction of laboratory set up is easy.
- No need for implantation of electrodes to the brain.
- Kindling protocol can be easily repeated among laboratories and species.
- Mortality rate is low. Most of the animals continue generalized motor seizures.^[47]
- PTZ kindling is also a model of partial epileptic disease as electrical kindling.^[16]

Conclusion

None of the animal epilepsy models can mimic epilepsy in humans perfectly when applied alone.^[48] Kindling model is among the first used models for AED discovery.^[9] PTZ is a GABA-A receptor antagonist,^[49] therefore tests/reference molecules acting via GABAergic mechanism are more effective in this model.^[20] In conclusion it can be considered that it is not possible to observe objectively all molecules which have antiepileptic nature with this method.

In this method, while the seizure scores are determined by direct observation, researcher should observe a limited number of animals since indicators of score 1 or 2 can be easily overlooked. In addition, room should be in appropriate temperature and kept silent. Otherwise, low room temperature and noisy environment may mislead the observer for concluding responses of animals to such stimulators as a convulsion score.

Win-PTZ model is presented as a novel approach for kindling protocol. Since it is a quite new method some aspects of this model needs further research and progress: Win-PTZ kindling model has not been assessed yet for GABA and glutamate binding alterations.^[23] If further affirmative results will be obtained, Win PTZ model can be presented as a more preferable method for time, effort and ethical aspects because it generates kindling phenomenon with less PTZ injections compared to the standard model. Epilepsy and models attempting to clarify its nature as well as studies aiming to find new molecules to cure the disease will be on the focus of scientists dealing with subjects of neuroscience.

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