Review Article

A Review on Chemical Induced Kindling Models of Epilepsy

Anil Kumar1*, Nidhi Sharma2, Manveen Bhardwaj1, and Sumitra Singh3

1Pharmacology Division, University Institute of Pharmaceutical Sciences, UGC Centre of Advanced Study, Panjab University, India
2Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science & Technology, India
3Department of Pharmaceutical Sciences, Panjab University, Chandigarh-160014, India, Email: kumonrips@yahoo.com

Abstract

A variety of animal models have been developed to study the effect of antiepileptic drugs. These models provide the means of inducing changes and causing alterations in neural function which may lead to epileptogenesis. Kindling is a lasting change in brain function caused by repeated stimulation resulting in an increased seizure duration and progressive intensification of seizure activity. The kindling model is widely used by investigators to provide insights into epileptogenesis. Kindled seizures can be induced in a number of animal species by electrical stimulation of brain as well as by chemical convulsants. The repeated administration of convulsant agents at sub-threshold concentrations is known as chemical kindling. This phenomenon can be achieved using convulsants like GABAergic antagonists, neurotoxicants, local anaesthetics etc. Chemical kindling has attracted the interest of many investigators as a model to study the effects of multiple seizures on the brain. The present review is an attempt to compare the various chemical models of kindling.

INTRODUCTION

Epilepsy is one of the major neurological disorders which are characterized by recurrent and unpredictable interruptions of normal brain function, called epileptic seizure. Epileptogenesis is the gradual process by which a normal brain develops epilepsy. Epilepsy is a chronic condition in which seizures occur. These changes to the brain occasionally cause neurons to fire in a hyper-synchronous manner. This hyper-synchronous firing of neurons is called seizures. The discovery and development of new antiepileptic drugs relies heavily on the preclinical use of animal models to establish efficacy and safety prior to first trials in humans [1]. A diversity of animal models is available for the study of epilepsy and these models have a proven history in enhancing our understanding of basic mechanisms underlying epileptogenesis [2]. The kindling model is widely used to study the epileptogenesis and discovery of antiepileptic drugs. Kindling is a lasting change in brain function that results from repeated focal stimulation and leads to the development of a predisposition to epileptiform convulsions [3]. It is associated with Tran’s synaptic changes, synaptic re-organization, long term potentiation, changes in synaptic morphology, protein synthesis and axonal transport [4]. It was invented by Goddard in 1967 and now this model has widely been accepted as a functional epilepsy model in which the altered neuronal response develops in the absence of gross morphological damage that is seen in many other epilepsy models [5]. It has become major focus of the neuroscientific research [6]. The achievement of the kindling criterion takes a long time, usually between 15 and 38 days, depending on the kindling procedure and animal strain [7]. The advantages of the kindling model for epilepsy research are clear: precise focal activation of the target brain sites is possible, development of chronic epileptogenesis is reliably observed, the pattern of seizure propagation and generalization is readily monitored, and interictal, ictal and postictal periods are easily monitored [5].

PHENOMENON OF KINDLING

Kindled seizures can be induced in a number of species, including rats and mice. It can be induced by electrical stimulation of different areas of brain such as the amygdala, hippocampus and frontal cortex [8,9]. It has been shown that an effect similar to electric kindling can be induced by the repeated administration of subconvulsant doses of central nervous system stimulants [10]. Different stages of seizures are observed during kindling. Racine’s grading of convulsive stages of kindling was based on electrical stimulation of amygdala which includes five stages. The stages 1 and 2 are primarily associated with facial and oral activities which include ipsilateral eye closure and blinking followed by head bobbing and drooling. Forelimb clonus eventually appears in the stage-3. Soon thereafter, in stage-4, the seizures generalize with stronger clonus and rearing. Then dramatic rearing and falling behaviour is observed in stage-5 [9].
Researchers continue to stimulate the animal in most kindling studies till the development of stage-5 seizures [11]. When more carefully examined, it should be observed that the amygdala kindled stage-5 seizures are not merely clonic but rather tonic-clonic seizures and involves all four limbs. Animal is considered as fully kindled if there is development of stage 5. This effect of kindling is long lasting and may endure for the life of animal [12]. Kindling starts with the limited neural circuits and with increased duration of seizures, changes in brain function occur [13].

**CHEMICAL KINDLING**

Kindling induced by repeated administration of convulsant agents at subthreshold concentrations is known as chemical kindling. These agents can induce kindled seizures by direct intracerebral administration or systemic administration. There are many chemicals which possess primary actions on central nervous system function. Some of these chemicals lead to appearance of convulsions with acute high dose exposure [14,15]. Administration of these chemicals in subthreshold doses prior to the electrical stimulation may also demonstrate their convulsant properties in a standard kindling paradigm [16]. However, these chemicals also produce kindling effect if delivered repeatedly at low concentrations in the absence of electrical stimulation [17-19]. Different chemoconvulsants used for kindling are shown.

**Kindling induced by GABAergic antagonists**
y-Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter which maintains the inhibitory tone to counterbalance the neuronal excitability. Seizures may be caused when this balance is disturbed. Therefore, GABAergic antagonism is a mechanism of action of some chemical agents that induce seizures [20]. Pentylenetetrazole, bicuculline, picrotixin and β-carbolines are potent GABA antagonists which are preferred to develop kindling models of epilepsy.

**Pentylenetetrazole-induced kindling:** Pentylenetetrazole (PTZ) has been widely used in experimental models of epilepsy. Absence, myoclonic and generalized tonic-clonic seizures are induced by PTZ administration. It is a commonly preferred behavioural approach used for chemical kindling to study brain excitability. PTZ has a proconvulsant effect on acute administration and induces convulsions in rats and mice [21]. After repeated injections the susceptibility of seizures was increased [22]. After every PTZ injection a seizure score is calculated [23]. Rapid and strong seizures are initiated 5 min after a single high dose of PTZ (50 mg/kg) in animals which lasts for approximately 30 minutes. After repeated daily administration of a convulsive dose of PTZ (30 mg/kg) the animals show very weak behavioural overactivity during the first 1-2 weeks. However, stronger epileptic activity is developed in the following 3-4 weeks, and finally leads to full kindling in 4-6 weeks [4].

Various behavioral, neurophysiological and neurochemical changes occur during PTZ-induced kindling. PTZ causes atrophy, selective neuronal loss and astrocytosis in hippocampus. Alterations in GABAergic systems, glutamergic systems and antioxidant defense systems have been observed in PTZ-induced animal models [24].

Kindling produced by PTZ may be related with permanent attenuation of inhibitory function of GABAergic system [21]. This activity is especially due to blockade of GABA_A-gated chloride receptors [4,25] GABA_A receptor number or function may be modified by either single or repeated PTZ administration. Injection of a moderate dose of PTZ which produces kindling, has several neurochemical effects including a decrease in GABA binding, [35S] t-butylicyclohexorphorionate (TBPS) binding and in GABA stimulated Cl⁻ uptake [26].

An alteration in density and sensitivity of different glutamate receptor sub types also occurs due to PTZ. An increase in the density of glutamate receptor, glutamate binding and hence increased glutamate concentrations in the hippocampus after PTZ kindling has been observed [27]. Pentylenetetrazole in toxic doses induces massive release of endogenous glutamate in various structures of the brain. Pentylenetetrazole-induced seizures in rats are associated with glutamate activation of AMPA and NMDA receptors in the brain [28]. It was reported that PTZ kindling phenomenon was sustained by AMPA receptors in cortex and basal ganglia. AMPA has also an important role in appearance of epileptiform burst discharges in hippocampal slices.

**Bicuculline-induced kindling:** Bicuculline is an alkaloid which produces generalized seizures after systemic administration. Unilateral application of bicuculline in deep prepiriform cortex is sufficient to induce generalized seizures [29]. The bicuculline kindling is identical to electrical kindling in many respects. A similar pattern of seizure development has been observed which eventually results in general convulsive seizure. However, the rate of chemical kindling caused by bicuculline is much faster than the electrical kindling. Multiple seizures are caused by a bicuculline injection in the kindled state [30]. The changes in arterial blood gas concentrations, cerebral blood flow and extracellular ionic concentrations have been extensively studied [31]. The kindling effect of bicuculline persists semi-permanently and the phenomenon is not correlated with visible tissue damage at the stimulating site. Bicuculline is a GABA_A antagonist, and thus amygdala neurons bearing GABA_A receptors may play a very important role in the initiation and development of amygdaloid seizures. Bicuculline is supplied as either the free base or methyl derivative [32]. Bicucullinemethiodide is the methylated form of bicuculline which is more active than the parent drug molecule [33,34]. Repeated injection of bicuculline methiodide into the rat amygdala induces chemical kindling [35]. Bicuculline methiodide completely suppresses the fast inhibitory postsynaptic potential without affecting the slow hyperpolarization. At the same time, augmentation of both the fast and slow excitatory postsynaptic potential is caused by bicuculline leading to burst discharges [36].

**β- Carboline induced kindling:** β-Carboline alkaloids are a large group of natural and synthetic indole alkaloids [37]. β-Carbolines are identified as potential endogenous ligands for the benzodiazepine binding site, and found to have competitive affinity for benzodiazepine binding sites [38]. The β-carboline family of compounds have since been found tospan the complete range from full agonists to full inverse agonists at the benzodiazepine allosteric site for the GABA_A receptor [39]. FG-7142 (N-methyl-p-carboline-3-carboxamide) is an amide
derivative of β-carboline-3-carboxylic acid ethyl ester [39,40]. It is used as a proconvulsant and found to potentiate the kindling effect of a subconvulsive dose of PTZ as a negative ligand of benzodiazepine receptor [41,42]. Repeated administration of FG 7142 produces sensitization to its effects so that full seizures develop [43]. DMCM (6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate methyl ester and β-CCM (methyl β-carboline-3-carboxylate) are full inverse agonists of GABAβ subunit benzodiazepine receptor complex and thus are characteristic β-carbolines with convulsant activity in vivo [44,45].

**Picrotoxin induced kindling**: Picrotoxin is a poisonous crystalline compound, found in the fruit of the plant *Anamirta cocculus* (Moosseed family). Picrotoxin exerts its epileptogenic effect by blocking GABA-mediated chloride conductance. Various investigations suggest that picrotoxin kindles seizures by reducing the GABA inhibitory effect. Thus, repetitive excitatory events occur leading to paroxysmal depolarizing shifts [46]. Mechanism of picrotoxin inhibition of this receptor is a complex phenomenon. The inhibitor has been considered to be a simple open-channel blocker [47,48]. A mixed/noncompetitive inhibitor, or a noncompetitive inhibitor that binds to an allosteric site to stabilize a closed or desensitized state of ligand-gated ion channels [49,50]. Detailed analysis of single-channel current recordings suggested a more complex scheme [49]. Neither picrotoxin nor its more active component picrotoxinin had any effect on the conductance of single-channel events mediated by GABA† receptors [49,51,52]. Single-channel current recordings showed that picrotoxin decreased the channel-opening frequency in a manner compatible with the stabilization of an agonist-bound closed state that perhaps corresponds to a desensitized state [49]. Some investigations have suggested that endosulfan sulfate, the main metabolite also contributes to the acute endosulfan neurotoxicity. Endosulfan has been reported to compete for binding of GABA at the t-butylic bicuculline phosphothionate site (TBPS) [17,62,65,66].

**Lindane induced kindling**: Lindane (γ-hexachlorocyclohexane) is a chlorinated hydrocarbon pesticide [67]. Joy and colleagues reported the proconvulsant properties of lindane using an electrical kindling paradigm. In this kindling paradigm, repeated low doses of lindane was delivered prior to each daily electrical kindling stimulation resulting in accelerated rate, generalized seizures development and prolongation of the electrographic seizures accompanying each kindling stimulus [68]. The mechanism of action of lindane includes its binding to the GABA receptor ionophore complex which results in the disturbed effect of GABA and GABAergic neurotransmission [69,70]. Lindane stereospecifically binds to the 1-[35S]-butyl bicyclophosphorothionate (TBPS) site on the GABA receptor/ ionophore complex [71] and inhibits GABA induced Cl influx [66].

**Effect of various chemoconvulsants on GABAergic system**

**Trimethylolpropane phosphate induced kindling**: Trimethylolpropane phosphate (TMPP) is an ethyl bicyclophosphorothionate convulsant. It is produced during the partial pyrolysis of certain synthetic ester-based turbine lubricants supplemented with phosphate-based lubricity additives. Repeated exposure to subconvulsive doses of TMPP results in facilitation of the electrical kindling of amygdale [72]. It mimics the responses shown by the GABAA antagonist bicuculline. The studies suggest that TMPP acts by a competitive antagonism of GABA inhibitory function. Binding assays have revealed that binding of TMPP to the GABAA receptor complex occurs with more affinity than picrotoxin, but less affinity than tert-butyl-bicyclo-[2.2.2]-phosphorothionate (TBPS). The epileptogenic effects caused by TMPP are consistent with this binding [61,73-75]. Furthermore, the benzodiazepines have been found effective against TMPP by increasing the time of seizures occurrence and reducing the severity of generalized convulsions [61,74-76]. These effects of benzodiazepines have also been observed against trimethylolpropane phosphothionate, a structurally related compound. Some investigations have shown that TMPP blocks the Cl current in hippocampal neurons [74,75,77].

**Kindling induced by local anaesthetics**: The use of local anaesthetics in excessive doses caused occurrence of convulsions in humans. Previous exposure to these agents or other kindling substances has been a predisposing factor. Thus, the local anaesthetics have been attributable to the kindling process. Kindling seizures produced by these agents seems to be affected by the dose and frequency with which these agents are administered for kindling to occur [78].

**Cocaine induced kindling**: Cocaine (benzoylmethylecgonine) is a CNS stimulant and found in the leaves of *Erythroxylon coca* (*Erythroxylaceae*), trees that are indigenous to Bolivia and Peru (WHO, 2004). Multiple forms of toxicity are associated with cocaine abuse and seizures represent one of the major fatalities.
induced by cocaine. In chronic cocaine abusers, kindling has been suggested as a possible mechanism for seizures [79]. Cocaine induce upregulation of cortical NMDA receptors. The findings suggest that cocaine induced kindling is associated with upregulation of striatal, amygdala and hippocampal NMDA receptors. However, the maintenance of kindling depends upon the increase in NMDA receptor binding in amygdala and hippocampus [79]. NMDA receptors are involved in the process of sensitization to behavioral and convulsive effects of cocaine. Activation of the NMDA receptors also leads to the brain nitric oxide synthase (NOS) stimulation which is entailed in the induction and expression of cocaine kindled seizures [80]. Furthermore, expression and development of cocaine induced kindled seizures are inhibited by NMDA receptor antagonists [81]. Cocaine kindling also results in enhancement of the depolarization-dependent release of glutamate and enhanced glutamate neurotransmission which may play an important role in kindling process [82]. Long-lasting increase in sensitivity to convulsive effect is developed as a result of repeated administration of cocaine at high doses. Thus, cocaine induced kindling is a useful model to study sensitizing and epileptogenic effects of repeated cocaine administration and their neurochemical mechanisms [83,84].

Lidocaine induced kindling: Lidocaine induced seizures are qualitatively similar to the electrical kindling of amygdala [85]. It is suggested that the lidocaine induces kindled seizures in the limbic system. High doses of lidocaine cause alteration of limbic system excitability and ultimately seizure discharges are induced. Limbic structures activation caused by lidocaine is selective and marked activation is produced particularly in hippocampus and amygdala [86]. In high doses, lidocaine is excitatory and induces generalized convulsions [87]. Some studies suggest that lidocaine induced seizures by binding to the GABA-ionophore receptor complex [88].

LIMITATIONS OF THE CHEMICAL KINDLING

Although it is very beneficial to use the chemical kindling but there are some limitations of this model. Like as compared to electrical kindling chemical kindling model has the less experimental control on the timings of evocation of seizures. Delay in time occurs from the time of drug delivery to the onset of clinical signs of seizures. A care should be done there in between the interval of the stimulations so that there should be less accumulation of drugs.

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