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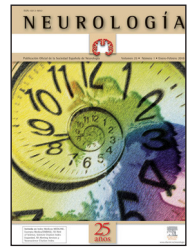

REVIEW ARTICLE

Experimental models in epilepsy

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Abstract
Introduction: Epilepsy is one of the neurological pathologies with the highest rate of incidence and with a significant number of negatives consequences. Current pharmacological treatments have an antiepileptic effect, allowing control over 70% of the patients, but they are not able to prevent the development of Epileptogenesis from occurring.
Method: We have reviewed the most relevant publications for experimental animal models with epilepsy by using the PubMed data base.
Results: We found a large number of publications related to different kinds of experimental models, both genetic (transgenic, genetically determined) and lesional, which appeared to resemble the different types of human epilepsy.
Conclusions: Even though many important improvements have been accomplished in the area of epilepsy in the last decades, there are still many aspects to be clarified. In this regard, experimental models might become a very useful means for a better understanding of pathophysiological mechanisms and in the search for more efficient treatments.

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PALABRAS CLAVE
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Epileptogénesis

Modelos experimentales en epilepsia

Resumen
Introducción: La epilepsia es una de las enfermedades neurológicas más frecuentes, y además conlleva una tasa de consecuencias negativas muy importante, tanto para el paciente como para los familiares. Su manifestación clínica principal es la aparición de crisis epilépticas recurrentes, que en el 70-80% de los casos se controlan con la medicación. Sin embargo, a pesar de que van apareciendo nuevos fármacos para el control de las crisis, no disponemos todavía de fármacos que consigan evitar la epileptogénesis.
Método: Revisamos las publicaciones más relevantes de modelos animales experimentales en epilepsia utilizando para ello la base de datos de PubMed.
Resultados: Se han encontrado un amplio número de publicaciones sobre tipos de modelos experimentales tanto genéticos (transgénicos, genéticamente determinados) como lesionales (químicos o eléctricos), que intentan imitar los diferentes tipos de epilepsia en humanos.
Conclusiones: A pesar de que en las últimas décadas se han hecho importantes avances en el campo de la epilepsia, aún quedan muchos aspectos por dilucidar. En este sentido, los modelos experimentales pueden suponer una herramienta muy útil para el avance en el conocimiento de los mecanismos fisiopatológicos y en la búsqueda de tratamientos eficaces.

Introduction

Epilepsy is a brain disorder which is clinically manifested, among others, by recurrent paroxysms. In its latest review, the International League against Epilepsy (ILAE) defined epilepsy as a brain disorder characterised by an enduring predisposition to generate epileptic seizures and by the neurobiological, cognitive and social consequences of this condition[1].

According to the WHO, the current prevalence is of 0.5-1% with an age-adjusted incidence of 30-50 cases/100,000 population per year; it is more common in people under 20 years and over 60 years[2]. Epileptic crises can be of very different nature and reflect the dysfunction of a cerebral cortex region. Depending on the area affected, the crises will present motor, sensory, autonomic or mental symptoms, associated or not with altered consciousness.

Diagnosis is mainly clinical, although the information from neuroimaging and EEG is essential for diagnostic confirmation. Approximately 30% of epileptic patients are not controlled with medication, so it is necessary to evaluate other treatment options (vagus nerve stimulation, surgery, ketogenic diet, etc.)[3]. Furthermore, epileptic patients, especially those with poor medication control, present a higher comorbidity, especially psychiatric (cases of depression, anxiety, psychosis), along with greater labour and social difficulties[4,5].

The high prevalence of the disease as well as the absence of an antiepileptogenic treatment, require further studies and the establishment of experimental animal models that allow us to understand the basic pathophysiological mechanisms and identify effective treatments.

Epileptic crises. Epileptogenesis

Epileptic crises are the manifestation of a transient cerebral function disorder characterised by an abnormal discharge, excessive and synchronous, of a group of neurons. Epileptogenesis is the phenomenon by which a normal neural network becomes hyperexcitable and is capable of generating spontaneous epileptic crises. Epileptogenesis may be genetic (as in the case of idiopathic or primary epilepsy) or acquired.

Over the years we have seen that adequate neuronal communication requires a balance between the excitatory and inhibitory signals received by neurons. Therefore, if this state is unbalanced, either by excessive excitation or by an inhibition defect, the result is a neuronal hyperexcitability that can lead to the onset of epileptic crises. Most of these crises are generated in cortical structures, although some subcortical structures, such as the thalamus, may be involved in some types of epilepsy.

A pathophysiological hypothesis of epilepsy is that alterations in inhibitory systems are the main causes of crisis onset, and a key role is therefore attributed to the interneurons that secrete the neurotransmitter GABA (gamma-aminobutyric acid)[6]. The experimental model of knockout mice for the DLX gene supports this theory. Deletion of this gene represents a selective loss of two subpopulations of interneurons (producers of the calcium binding protein calretinin and the neuropeptide somatostatin), which leads to the joint appearance of epileptic crises, spike-wave activity and mossy fibre proliferation[7].

The best known pathophysiological bases, both in animals and in humans, are currently those responsible for creating partial crises, followed by those generating absences.

Types of experimental models

Currently, an animal model is considered valid when it is able to reproduce a number of clinical features, totally or partially, that can be transferred to humans or other animal species.

In epilepsy we have models for acute and chronic epilepsy. Those for acute epilepsy are induced by administration of convulsive drugs or electrical stimulation. Chronic epilepsy models require more care, work and economic cost, but it has been shown that they reproduce the pathophysiology of epilepsy in humans better. Both types of models reproduce partial and generalised seizures; however, since epilepsy is characterised by the appearance of recurrent crises over time, only the models that reproduce this condition are considered as valid models of epilepsy.

Experimental models have been classified according to the type of crises that they can reproduce, as indicated below.

Models for focal seizures

The main regions of the brain studied as epileptic foci have been the neocortex, the hippocampus and the amygdala, although there are other susceptible areas, such as the inferior colliculus (involved in audiogenic epilepsy) and the olfactory bulb[8]. Experimental models of focal epilepsy reproduce focal motor crises fundamentally because, semiologically, they are very similar among different animal species and are easier to provoke than other types of focal seizures such as those in which consciousness or language is impaired.

The development of these types of models follows three basic steps:

—Initial aggression: this consists of causing an injury with the potential to generate a crisis (stroke, head trauma, status epilepticus, etc.). These models can be associated in some cases with acute symptomatic crises, whose pathophysiology is unknown, although it is presumed that several factors are involved; examples of factors are changes in the blood brain barrier, release of excitotoxins, such as glutamate and free radicals, and energy metabolism disorders[9]. The relationship between acute symptomatic crises and their role in epileptogenesis has not yet been clearly established.
—Latent period: after the initial aggression, there is a latency period with absence of crises, during which a number of structural and/or functional changes take place, leading to a situation of hyperexcitability (epileptogenesis).
—Chronic period: period of spontaneous recurrent crises.

Among the models for focal crises we can distinguish:

Models for partial motor/sensory crises

There are several animal models, among which we highlight:

1. Topical application of metals to the sensory or motor cortex: model of cobalt, aluminium or iron derivatives, among others. After a latency period of 1-2 months, crises appear that are characterised by shaking, contralateral to the lesion. The anatomical pathology shows gliosis and dendrite alteration.
2. Focal cryogenic injury: the production of this type of lesions has been used to assess the consequences of neonatal events. The result after its implementation in newborn rats is a lesion similar to polymicrogyria with *in vitro* hyperexcitability.
3. Topical application of convulsive substances: bicuculline, penicillin, picrotoxin.
4. Acute electrical stimulation.

Models for medial temporal lobe epilepsy

There are different experimental models for studying medial temporal sclerosis; of these, the kindling and status epilepticus models are the most commonly used. Both have in common the ability to induce a chronic epileptic state but differ in the process of epileptogenesis:

1. Kindling phenomenon: these consist of repeated stimulation, electrical or chemical, of various structures of the limbic system (usually amygdala, cortex and hippocampus), so that over time there is an increased excitability and the neurons become "pathological neurons" capable of generating epileptic crises, first, when they are stimulated and, subsequently, in some animal models, also spontaneously. The establishment of kindling is gradual and a series of stages can be distinguished, ranging from 0 to 5; during this last stage, the crises are permanent[10]. The initial mechanism of kindling is considered to be long-term potentiation (LTP), whose goal is to enable a brief discharge with repetitive frequency to produce a sustained synaptic response increase in the hippocampus that can last for days or weeks. The anatomical and biochemical changes observed in both kindling and in the repeated seizures or convulsive status are:

   —Release of glutamate that activates NMDA receptors.
   —Increase in intracellular calcium that activates protein kinase II, dependent on calmodulin.
   — Apoptosis and selective neuronal death in hippocampus areas CA1, CA3 and hilus. The increased susceptibility of these areas that leads to apoptosis is due to two factors: lack of protective intracellular mechanisms and a greater proportion of receptors with affinity for excitatory amino acids (glutamate and aspartate)[11].
   —Proliferation or sprouting of axons of dentate fascia granule cells (mossy fibres) that make contact with the molecular layer of the dentate gyrus neurons, both with excitatory neurons and with inhibitory interneurons. The role of mossy fibre innervation in epileptogenesis remains in dispute, and it has been indicated that it may be more a consequence than the

cause of the crises. However, there are other studies that show how this proliferation amplifies discharges[12].

— Increase of neurogenesis in the dentate gyrus[13,14]. These newly formed neurons have different electrophysiological properties and locations from the usual, so it is assumed that they are involved in generating abnormal hyperexcitable networks and in the emergence of new epileptic crises[15,16].

2. Status epilepticus model: in this case different convulsive agents, such as kainic acid and pilocarpine, are applied through systemic or intracerebral injection. This results in an acute status epilepticus with generalised tonic-clonic seizures, which constitute the initial event. After this episode, there is a latent phase that is crisis-free and that may last for weeks. After this, spontaneous and recurrent crises begin (chronic phase). Anatomical studies of the hippocampus of rats subjected to this method show alterations very similar to those of the human medial temporal sclerosis with neuronal death and astrogliosis in the hippocampus and amygdala[17]. Obtaining these experimental models allows us not only to observe if the anatomopathological changes are similar in the late phase, but also to analyse the changes that occur in early stages and during epileptogenesis.

Models for posttraumatic crisis

Different types of animal species (rat, cat, guinea pig) have been used for the study of posttraumatic crises, generating crises with intracortical blood injection. Although the exact mechanism is unknown, one of the accepted hypotheses is that the deposit of blood, and its subsequent transformation into iron deposits and derivatives, leads to ATPase inhibition of the NA^+/K^+ pump due to the ability of iron to bind to ATP. The malfunction of this ion transporter generates a change in the electrical charge of the membrane and increases neuronal excitability. In addition, iron can also react with lipids from the cell membranes and lead to the emergence of free radicals that induce membrane lipid peroxidation[18,19].

It is also assumed that the development of posttraumatic epilepsy, like other types of epilepsy, might have a genetic background related to the number of inhibitory interneurons that exist in each individual depending on their genetic background and, in particular, on the number of GABAergic interneurons (chandelier neurons) in the cerebral cortex and the hippocampus. These chandelier cells, through numerous collaterals, inhibit projection neurons from the output of the (where inhibition is maximal). These cells are thus thought to be decisive in the development of both spontaneous epilepsy and epilepsy secondary to brain lesions (since neuronal death occurs preferentially in interneurons)[20,21]. Therefore, the greater the number of interneurons in an individual (genetically determined), the more inhibition is facilitated and the more the development of crises is hindered when there are brain injuries.

Models for generalised crises

It is currently considered that genetic susceptibility is involved in at least one-third of epilepsy cases in humans. Although most generalised epilepsies have complex inheritance patterns, some have a Mendelian pattern with simple genetic mutations. Many of these mutations have been found in genes encoding ion channels (channelopathies), although genetic alterations may also cause abnormalities in neuronal migration or degenerative conditions where epilepsy is another manifestation of the syndrome, such as tuberous sclerosis (altered tuberin gene), Lafora disease or Unverritch-Lundborg disease.

A better understanding of the biological aspects, environmental requirements and genome of the mouse, make this the species from which most of the genetic models are derived. In the study of generalised epilepsies, we distinguish two types of models:

1. Genetically modified (GM) models: knockout mice are used, in which a gene known for its role in neuronal excitability is deleted[22]. Most of these genes encode protein subunits that are part of the structure or function of voltage-dependent ion channels (sodium, potassium, calcium) or of channels associated to neurotransmitter receptors ($GABA_A$, AMPA/KA and NMDA). Some of these models are: mice without KCAN1 (it encodes a potassium channel and its absence prevents repolarisation, producing spontaneous crises and hyperexcitability of the hippocampus), mice with a *GABRB3* gene mutation (it affects $GABA_A$ receptor subunits and produces crises very similar to those of Angelman syndrome) or the mouse model without KCNQ2 (which attempts to replicate type 1 benign neonatal epilepsy)[23,24]. The problem with transgenic models is that, first, the results obtained in the laboratory can result in phenotypes that present certain differences with epilepsy in humans, indicating that the involvement of other genes (genetic background) intervenes in the final phenotype, and second, that different mutations may lead to the same epileptic phenotype.
2. Genetically epileptogenic models: these are animals that have, as a hereditary trait, the ability to present epileptic crises. This type of model is obtained by identifying the locus where the anomaly is located and then exploring the adjacent genes to identify the mutated gene and the mutation itself. There are different strains that allow us to study different types of epilepsy: *stargazer* mouse (alteration in the *CACNG2* gene, used as a model for absence), *lethargic* mouse (*CACNB1* gene mutation; it manifests with stoppage reactions), *tottering* mouse (*CNA1A* gene mutation, produces absences and convulsions), *weaver* mouse, etc.[25].

Among the animal models that present generalised crises we can distinguish:

1. Absences. One of the species used for the study of this type of crisis is the Strasbourg rat (GAERS rat) or the rat model WAG/Rij from Nijmegen. This model was designed by Marescaux et al. and presents spontaneous and continuous spike-wave discharges at 7-11 Hz in the EEG record, with clinical features similar to those of absences in adults[26]. Absences are characterised by abnormal oscillatory rhythms in thalamocortical circuits, whose

origin is in the inhibitory GABAergic neurons of the reticular nucleus of the thalamus. The ability of this nucleus for discharges is determined by the T-type calcium channels, which are activated by thalamic hyperpolarisation, generated in turn by inhibitory postsynaptic potentials originating in the $GABA_B$ receptors. The hypothesis that a $GABA_B$ receptor hyperfunction is involved in the genesis of absence has been raised from studies in rats where it was found that the $GABA_B$ receptor inhibitors suppressed the absences, while the agonists (tiagabine, vigabatrin) exacerbated them.

2. Reflex crises. These crises are produced by known stimuli, both external and internal. It is supposed that there is a functionally abnormal zone of the cerebral cortex with a decreased excitability threshold, which generates crises when activated by an external or internal stimulus. The aetiology of reflex crises is very diverse, but we can distinguish groups such as those caused by lesions and those that are genetic[27]. The mandrill of the species *Papio papio* is a primate sensitive to photic stimulation in 20 to 30 Hz; in response to this stimulus, it presents a facial and cervical myoclonus reflex that can end in generalised tonic-clonic seizure. This primate constitutes a model of reflex epilepsy, in which genetic alterations produce a hyperexcitability of the cortical area responsive to visual stimuli, which then becomes the area of origin of the initial electrical activity[28]. The photoparoxysmal response (PPR) (appearance of epileptiform activity during light stimulation) indicates an underlying genetic trait of multifactorial inheritance, with age-dependent expression. One of the genes that could be involved is the one that codes for subunit 1 A of the sodium channel (SCN1A), whose mutation causes severe myoclonic epilepsy in infancy, since these patients present PPR in more than 90% of cases[32]. Other genes involved might be those associated with progressive myoclonic epilepsies, such as Unverricht-Lundborg disease (*EPM1* gene) or Lafora disease (*EPM2A* and *EPM2B* genes), although there are currently no studies that have been able to demonstrate this relationship. A second model of interest in reflex epilepsies is that of the DBA/2 mouse and also the genetically epilepsy-prone rat (GEPR rat). Both present epileptic crises with auditory stimuli, so they can serve as experimental models for audiogenic epilepsy[29]. Studies carried out on both models show a subcortical origin of the crises, so they could reveal different pathophysiology from that of other reflex epilepsies[30]. Among the findings published, there are studies that have shown morphological changes in brainstem auditory nuclei of some of these animals[31].
3. Generalised tonic-clonic crises. In this type of crisis, the underlying factor is assumed to be an inhibitory tone decrease or an excitatory tone increase, which facilitates discharge synchronisation and propagation. Thus, either spontaneously or by physiological stimuli, a synchronised discharge is started in the cortex, which then propagates to the thalamus where it is amplified until it reaches the brainstem and causes seizures and generalised rigidity. To reproduce this type of crisis in animals, substances that promote epileptic crises by decreasing GABAergic tone (picrotoxin) or increasing glutamatergic tone (kainic acid) are systemically administered. In generalised tonic-clonic seizures, when Fos protein (encoded by the *c-Fos* gene, one of the first activated after postsynaptic stimulation) is used as a neuronal marker to assess areas involved in seizures, a diffuse distribution of the protein can be observed in the cortex and hippocampus, but not in the thalamus[33].

Experimental models in status epilepticus

Status epilepticus (SE) has classically been defined as the crisis which lasts longer than 30 min or as the situation in which two or more seizures occur without a recovery of consciousness between them. However, new definitions have recently been proposed in which SE is considered when the crisis does not subside within 5 min[34,35]. SE represents a medical emergency with a mortality rate of 40%[36].

The design of experimental models for SE attempts to explain the pathophysiological mechanisms that produce it and the causes of secondary neurological deficits. Such models also try to find effective treatments that allow us to reduce its morbidity and mortality. To obtain an SE model, animals are subjected to chemical agents (systemic administration of kainic acid or pilocarpine) or to electrical stimulation (in the amygdala or hippocampus) until a status epilepticus is reached. The creation of these models has enabled us to reach the following conclusions:

- Between 30 and 60 min after the onset of status epilepticus, failure of the homeostatic mechanisms[37] begins to take place.
- Neuronal death may be caused by an excess of glutamate, which activates postsynaptic NMDA receptors and allows massive calcium influx into the cell, thus giving rise to excitotoxicity phenomena[38].
- Neuronal death after SE does not occur immediately, so there is a period in which neuroprotective agents may be administered to avoid neuronal damage. To this end, there have been studies in rats that were administered NMDA receptor antagonists (MK 801) or valproic acid after an SE. It was found that damage to the hippocampus was less than in controls, but there were no changes in epileptogenesis or in the occurrence of subsequent crises[39].
- Once the SE is maintained over time, GABAergic drugs and benzodiazepines are less effective than substances that inhibit glutaminergic neurotransmission. This is because benzodiazepines and GABAergic drugs lose their effectiveness when some of the functional properties of hippocampal $GABA_A$ receptors are altered. One hypothesis is the internalisation of these receptors in endosomes (thus inactivating their function) and the externalisation of NMDA receptors[40,41].
- SE induces an increase in the expression of transporter proteins, such as P-glycoprotein (Pgp), in the blood-brain barrier. This protein inhibits brain reuptake of many lipophilic compounds (such as phenobarbitpl and phenytoin) so their increase results in a decrease in the

brain concentration of these drugs. This protein increases after 24 h, so some authors do not consider it to have a crucial role in SE[42]. It has been shown in animal kindling models that one of the alterations induced is Pgp overexpression, which would justify the low response observed to treatment with antiepileptic drugs (AEDs) with different mechanisms of action[43].

Experimental models and treatments

Experimental models in animals have also been used to assess new treatment efficacy and toxicity before undergoing clinical trials. The disadvantage of these models in the study of epilepsy is the difference between species (mainly in metabolism and difficulty in assessing cognitive functions) and the wide variety of epileptic crises in humans that makes it impossible to represent all of them. Among the epileptic models most frequently used to assess new treatments are:

- —Maximal electroshock test (MET): this consists of applying a maximum electrical stimulation that can generate seizures. It represents a model of acute crises, sensitive to compounds such as phenytoin, which work by modulating the activity of the voltage-dependent sodium channels. However, this test is not a perfect model because it can give false negatives in drugs with a different mechanism, such as levetiracetam, tiagabine or vigabatrin.
- —Subcutaneous pentylenetetrazole (PTZ) test: this identifies useful drugs in absences and myoclonus. This test is being used increasingly less often due to conflicting results obtained in these models with respect to humans. For example, lamotrigine, which is a useful substance in absences, has been ineffective in this test; or drugs such as tiagabine or vigabatrin that, having shown their success in this model, have proved useless or even capable of worsening absences in humans. Currently, it is considered that genetic models such as the GAERS rat or the lethargic mouse are better predictors of the clinical efficacy of a compound against absences than the PTZ model.
- —Electrical kindling model: it allows us to study neuroplasticity and identifies compounds useful in medial temporal lobe epilepsy. This model has been used to assess drugs such as lacosamide, which has shown not only an antiepileptic activity, but also the possibility of delaying kindling-induced epileptogenesis[44].

The AEDs currently available are useful to control crises, but overall we have not been able to demonstrate clearly that they are antiepileptogenic. There are some studies that, in isolation, raise the possibility that certain AEDs (including valproate, topiramate, phenobarbitol and, recently, levetiracetam) can be considered as preventive treatments[45-48].

In addition, the observations in animals have shown that vagus nerve stimulation (VNS) offers efficacy in the control of seizures induced electrically and chemically[49]. Although the mechanism underlying the anticonvulsant effect is unknown, the results indicate several points of action: lower cortical excitability by decreasing glutamate receptors, increased GABA receptors in the reticular nucleus of the thalamus and alteration of neural synchronisation with frequencies above 25 Hz[50,51]. VNS could also have a prophylactic effect in epileptogenesis. This is suggested by studies carried out in models of amygdaloid electrical kindling in cats, in which it was observed that VNS pretreatment caused a delay in the generalised spread of convulsive activity, which remained in the initial kindling stages (I-III).

Another model has been that of transgenic $Tsc1^{GFAP}CKO$ mice, designed to assess substances such as rapamycin in tuberous sclerosis treatment.

Conclusions

Despite the many advances that have taken place in the field of epilepsy in recent years, there are still great unknowns to be clarified. From this point of view, experimental models in animals may represent a very useful tool, both in elucidating the pathophysiological mechanisms of epilepsy and in identifying treatments, not only with antiepileptic effect, but also antiepileptogenic. There are a great variety of models that try to represent the different types of epilepsy that exist in humans (idiopathic camouflaged by transgenic models, symptomatic through electrical or chemical creation of a potentially convulsive injury); each model presents a number of advantages and disadvantages, but the ideal model has not yet been found. At present, we select it depending on the design and purpose-target of the study.

In conclusion, we can say that further research in epilepsy is needed, as well as the use of valid experimental models. This is not only due to the high prevalence/incidence of this disease, but also because of the lack of effective treatments and the devastating consequences that it can have on the patients and their families.

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