New insights from the use of pilocarpine and kainate models

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Abstract

Local or systemic administration of pilocarpine and kainate in rodents leads to a pattern of repetitive limbic seizures and status epilepticus, which can last for several hours. A latent period follows status epilepticus and precedes a chronic phase, which is characterized by the occurrence of spontaneous limbic seizures. These distinct features, in a single animal preparation, of an acute damage induced by status epilepticus, a silent interval between injury and the onset of spontaneous seizures, and a chronic epileptic state have allowed antiepileptic drug (AED) studies with different purposes, (a) in the acute phase, identification of compounds with efficacy against refractory status epilepticus and/or neuroprotection against damage induced by sustained seizures; (b) in the latent period, identification of agents with a potential for preventing epileptogenesis and/or against seizure-induced long-term behavioral deficits and (c) in the chronic phase, testing drugs effective against partial and secondarily generalized seizures. Studies on pilocarpine and kainate models have pointed out that some AEDs or other compounds exert an antiepileptogenic effect. The analogy of the latent phase of pilocarpine and kainate models with the acquisition of amygdala kindling should encourage testing of drugs that have proved to suppress the evolution of amygdala kindling. Drug testing in the chronic phase should not address only the suppression of secondarily generalized motor seizures. Most of current tools used to quantify spontaneous seizure events need to be coupled to electrophysiology and more sophisticated systems for recording and analyzing behavior. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Pilocarpine and kainate models replicate several phenomenological features of human temporal lobe epilepsy and can be used as animal preparations to understand the basic mechanisms of epileptogenesis (Ben-Ari, 1985; Turski et al., 1989; Buckmaster and Dudek, 1997). Local or systemic administration of pilocarpine and kainate in rodents leads to a pattern of repetitive limbic seizures and status epilepticus, which can last for several hours (Cavalheiro et al., 1982; Turski et al., 1983; Leite et al., 1990; Furtado et al., 2002). A somewhat variable latent period follows status epilepticus and precedes the chronic phase, which is characterized by the occurrence of spontaneous limbic seizures. The brain damage induced by
status epilepticus in such preparations may be considered an equivalent of the initial precipitating injury event, usually a prolonged febrile convolution, which is commonly found in patients with mesial temporal lobe epilepsy (Mathern et al., 1996).

Neuropathological changes such as neuron loss in several hippocampal subfields and reorganization of mossy fibers into the molecular layer of the fascia dentata are observed in both models and are similar to hippocampi from patients with hippocampal sclerosis (Tauck and Nadler, 1985; Sutula et al., 1989; Babb et al., 1991; Mathern et al., 1993; Mello et al., 1993; Mathern et al., 1995). This abnormal synaptic reorganization has been suggested to be an anatomical substrate for epileptogenesis (Tauck and Nadler, 1985; Babb et al., 1991; Buckmaster and Dudek, 1997).

The distinct features, in a single animal preparation, of an acute damage induced by status epilepticus, a silent interval between injury and the onset of spontaneous seizures, and a chronic epileptic state have allowed antiepileptic drug (AED) studies with different purposes, (a) in the acute phase, identification of compounds with efficacy against refractory status epilepticus and/or neuroprotection against damage induced by sustained seizures; (b) in the latent period, identification of agents with a potential for preventing epileptogenesis and/or against seizure-induced long-term behavioral deficits and (c) in the chronic phase, testing drugs effective against spontaneous recurrent partial and secondarily generalized seizures.

2. Methods of induction

2.1. Pilocarpine

The status epilepticus induced by pilocarpine can be achieved by high doses of intraperitoneal injections, usually over 300 mg/kg (Turski et al., 1983; Leite et al., 1990; Cavalheiro et al., 1991). Pre-treatment with lithium chloride 24 h prior to pilocarpine injection, at a dose of 3 mEq/kg, i.p., potentiates the epileptogenic action of pilocarpine and the amount of the drug can be reduced by ten times (Honchar et al., 1983; Jope et al., 1986; Clifford et al., 1987). Acute behavioral manifestations and the pattern of seizure-induced brain damage after high doses of pilocarpine and lithium–pilocarpine models are very similar; however, there is evidence that some antiepileptic compounds respond differentially to status epilepticus, suggesting that distinct biochemical mechanisms control seizures in these two preparations (Ormandy et al., 1989; Sofia et al., 1993). Chaudhary et al. (1999) have shown that lithium chloride pretreatment schedule can be adopted anytime between 2 and 24 h with results similar to the 24 h interval. It seems that the lithium–pilocarpine protocol reduces mortality, and avoids many of the peripheral cholinomimetic side effects of high doses of pilocarpine (Chaudhary et al., 1999). More recently it has been shown that lithium–pretreatment, followed by several low doses of pilocarpine, efficiently produces status epilepticus and chronic epilepsy in rats with much lower mortality rates than a single dose of pilocarpine (Glien et al., 2001). The reason for the reduced mortality is unknown, but it seems plausible that repeated low-doses intervals allow individual dosing with respect to inter-rat differences in sensitivity to the convulsant action of pilocarpine, which is not possible with the single-dose administration (Glien et al., 2001).

Local administration of pilocarpine delivered either intracerebroventricularly or directly into the hippocampus has been used in studies assessing the seizure-induced changes in amino acid levels and the effectiveness of some anti-epileptic agents (Croiset and De Wied, 1992; Millan et al., 1993; Croiset and De Wied, 1997; Smolders et al., 1997; Lindekens et al., 2000). In none of these studies a detailed behavioral, electrophysiological and morphological analysis of the model was made. More recently, however, Furtado et al. (2002) have shown that intrahippocampal-pilocarpine injection (2.4 mg/μl; injected volume 1.0 μl) induces status epilepticus with near zero mortality. Timm positive-mossy fiber sprouting and spontaneous recurrent seizures are also observed in intrahippocampal-pilocarpine injected rats, with similar seizure frequency than that observed in systemically injected animals (Furtado et al., 2002).
2.2. Kainic acid

Limbic status epilepticus in rats can be induced by kainic acid with either local administration (intracerebroventricular or intrahippocampal, at doses of 0.1–3.0 μg per hemisphere) or injected systemically (usually at doses of 15–30 mg/kg). Such treatment protocols have often been associated with a relatively high mortality rate and a low percentage of rats becoming epileptics (Nadler et al., 1980; Cavalheiro et al., 1982; Cronin and Dudek, 1988; Cronin et al., 1992; Mathern et al., 1980; Caçalheiro et al., 1982; Cronin and Mathern, 1988; Mikati et al., 1994). Hellier et al. (1998) proposed a modified treatment protocol using multiple low doses (5 mg/kg, i.p.) of kainate. This protocol had a relatively low mortality rate (around 15%) and nearly all kainate-treated rats (97%) had two or more spontaneous motor seizures months after treatment (Hellier et al., 1998).

3. Acute phase

Several drugs have proved to efficiently abort or attenuate status epilepticus when injected either before or right after pilocarpine or kainate administration. In fact, benzodiazepines and barbiturates have been broadly used to prevent the relatively high mortality associated with prolonged seizures in both models.

In the pilocarpine model, interruption of status epilepticus by pentobarbital and diazepam after different duration times indicates that early status epilepticus suppression can prevent spontaneous recurrent seizures. In addition, severe cell loss and synaptic reorganization are prevented if treatment is established with 30 min of status epilepticus (Lemos and Cavalheiro, 1995). In the intrahippocampal pilocarpine preparation, diazepam (4 mg/kg; i.p.) injected 90 min after sustained seizures can suppress status epilepticus and reduce acute mortality to nearly zero even though this protocol does not hamper the expression of spontaneous recurrent seizures and the presence of Neo-Timm positive mossy fiber sprouting (Furtado et al., 2002). Clonazepam, ED50 0.35 mg/kg (0.25–0.49), phenobarbital, 23.4 mg/kg (18.5–29.6), and valproic acid, 286 mg/kg (202–405), when administered before systemic pilocarpine, prevented the buildup of limbic seizures and protected against seizure-related brain damage. Pretreatment with trimethadione, 179 mg/kg (116–277) resulted in a moderate protection against pilocarpine-induced seizures, whereas diphenylhydantoin, 10–200 mg/kg and carbamazepine, 10–50 mg/kg, had no effect and did not prevent pilocarpine-induced brain damage (Turski et al., 1987). In the lithium–pilocarpine preparation, however, carbamazepine displays a protective effect while felbamate is far more effective than in animals treated with high doses of pilocarpine alone (Sofia et al., 1993). Both preparations seem to respond differentially to N-methyl-D-aspartate (NMDA) receptor antagonists. Pretreatment with MK-801 (dizocilpine), a noncompetitive NMDA receptor agonist, produces an effective dose-dependent anticonvulsant action in the lithium–pilocarpine model but not in rats treated with pilocarpine alone (Ormandy et al., 1989). In spite of the lack of effect in blocking the onset of status epilepticus in the pilocarpine model, MK-801 (4 mg/kg), given 20 min prior to pilocarpine, prevents later spontaneous recurrent seizures and protects against CA1 pyramidal damage (Rice and DeLorenzo, 1998).

In addition, MK-801 seems to have a synergistic effect with diazepam against refractory status epilepticus. Early pilocarpine-induced status epilepticus responds rapidly to diazepam treatment, whereas status epilepticus of longer duration turns increasingly less responsive to treatment. MK-801 pretreated rats respond rapidly to diazepam treatment, even after 60 min of status epilepticus, indicating that NMDA receptor activation plays a role in the seizure-induced refractoriness to benzodiazepines in status epilepticus (Rice and DeLorenzo, 1999). Ketamine, another noncompetitive NMDA receptor antagonist, at a dose of 100 mg/kg, i.p., exerts a time-dependent protection of hippocampal cells and prevents late spontaneous recurrent seizures and spatial memory deficits in the Morris water maze (Hort et al., 1999).

Studies on the kainate model have indicated that compounds with an antagonistic effect on glutamate receptors, both on NMDA and non-NMDA types, can have a protective effect on neurotoxic-
induced damage and reduce late spontaneous seizures and/or behavioral deficits, when given either before or right after kainate treatment. Stafstrom et al. (1993) have shown that MK-801 pretreatment prior to kainate-induced status epilepticus, while not substantially altering the acute epileptic behavior, reduces spontaneous recurrent seizure frequency and fluoroethyl seizure susceptibility. NBQX, an AMPA receptor antagonist, when given at different times after kainate treatment, at a dose of 30 mg/kg per dose, reduced the severity of status epilepticus. Later, animals receiving kainate and treated with NBQX did not show memory impairment or histologic damage in the CA1 and CA3 subfields, however, spontaneous recurrent seizure frequency was not different from non-treated animals (Mikati et al., 1999).

D-cycloserine, a partial agonist for the NMDA receptor-associated glycine-binding site, exerts a potent, dose-dependent and long lasting anticonvulsant effect against kainate-induced seizures. Contrary to MK-801 and diazepam, D-cycloserine did not significantly alter the number of automatisms (pot belly dogs shakes) induced by kainate (Baran et al., 1994). MacGregor and coworkers have shown that when the GABA-A agonist muscimol, dizocilpine (MK-801) and the adenosine A1 receptor agonist R-N6-phenylisopropyladensine are administered together with chloromethiazole, at their respective ED25 doses, a potentiation effect was apparent in the degree of neuroprotection. These findings suggest that the combination of neuroprotective agents with different mechanisms of action can lead to a synergistic protection against excitotoxicity (MacGregor et al., 1997).

Studies on the intrahippocampal kainate model have shown that treatment with phenobarbital (60 mg/kg, s.c., once daily) after kainate, for 5 days, suppresses seizure activity, protects against hippocampal excitotoxic damage, reduces mossy fiber sprouting and completely abolishes the increased susceptibility to kindling associated with kainic acid (Sutula et al., 1992). Vigabatrin (gamma-vinyl GABA, 1000 mg/kg, i.p.), given 24 h before kainate treatment, decreased neuronal damage in the CA3a and CA1 hippocampal subfields, attenuated the severity of seizures but had no effect on the development or generalization of convulsions (Halonen et al., 1995). Using higher doses (1500 mg/kg) Mecarelli et al. (1997) have shown that vigabatrin reduces the incidence of epileptic manifestations and the subsequent mortality.

Felbamate also seems to have some long-term neuroprotective effects after kainate-induced status epilepticus. Thirty-day-old rats treated with felbamate (300 mg/kg) 1 h after kainate and tested 50 days later have longer latencies to fluoroethyl-induced seizures and performed better on water maze, open field and handling tests (Chronopoulos et al., 1993).

4. Latent period

The importance of drug testing in the latent period has been stressed by many groups because there is a great deal of evidence that in this phase several molecular changes occur and may contribute to the process of epileptogenesis (Loscher, 1998). A putative compound with the property of inhibiting the process underlying the development of the epileptic condition may be considered a true antiepileptic or antiepileptogenic drug. In this sense, the latent period of pilocarpine and kainic acid models can be considered analogous of the acquisition or development phase of amygdala kindling in rats (Cavalheiro et al., 1991; Silver et al., 1991). Several drug studies in pilocarpine and kainate models initiate after the period of status epilepticus and last from few days to several weeks. Hence, such drug studies carried out in the latent period may overlap with the period of spontaneous recurrent seizures (chronic period).

Bolanos et al. (1998) compared in 35-day-old rats (P35) the long-term effects of valproate and phenobarbital after kainate. Phenobarbital, given from P36–75, did not have a protective effect on the prevention of learning impairment in the water maze, recurrent seizures or histologic lesions in the CA3, CA1 and dentate hilus. Valproate-treated rats, however, had no spontaneous seizures, no deficits in visuospatial learning and had fewer histologic lesions than animals receiving kainate alone (Bolanos et al., 1998). This antiepileptogenic effect of valproate confirms a previous study in the
amygdala-kindling model (Silver et al., 1991). In contrast, Pitkanen et al. (1999) have shown that vigabatrin, 1 or 2 h or 7 days after the beginning of kainic acid-induced status epilepticus did not prevent mossy fiber sprouting regardless of when treatment was started. Cilio et al. (2001) have shown that rats treated with gabapentin had a reduced incidence of spontaneous recurrent seizures, less intense pathological lesions and less aggressiveness when compared with saline treated controls.

There is a growing body of experimental data which suggests that there are differences between the mechanisms underlying epileptogenesis and the mechanisms involved in maintaining the expression of seizures once an epileptogenic process has been established. Therefore, it is reasonable to assume that drugs which prevent epileptogenesis may be quite different from drugs that prevent seizures (Silver et al., 1991; Shinnar and Berg, 1996; Loscher, 1998). Hence, the use of compounds that interfere on trophic factors which have impact on neuroprotection or damage-induced plastic changes, may also have an antiepileptogenic effect. Supporting this concept, Liu and Holmes (1997) have shown that administration of basic fibroblast growth factor for 7 days, starting 2 days before kainate, has neuroprotective effect, reduces spontaneous recurrent seizures, and prevents memory deficits on kainate-treated rats. In the pilocarpine model, local i.c.v. injections of antibodies against nerve growth factor suppress the cholinergic sprouting in the hippocampus but do not interfere with mossy fiber sprouting. The impact of this selective change on seizure frequency is unknown since a concomitant behavioral study is lacking (Holtzman and Lowenstein, 1995).

On the other hand, studies from Longo and Mello have indicated in pilocarpine and kainate models that cycloheximide, a potent protein synthesis inhibitor, was able to completely block mossy fiber sprouting without changing the frequency of spontaneous seizures (Longo and Mello, 1997, 1998). This finding would argue against a contributory role of mossy fiber sprouting on epileptogenesis. However, this finding has not been confirmed by Williams et al. (2000) in the pilocarpine model. The reasons for these inconsistencies are still unknown, but differences in rat strains might be a determinant (Longo and Mello, 1997, 1998).

5. Chronic period

It is surprising that there are not many studies of AEDs during the chronic phase of pilocarpine and kainate models. Most of the studies overlap with the silent period and were already mentioned in the previous section. In the pilocarpine model, AEDs that are efficient to suppress status epilepticus are not necessarily the same that are effective on controlling spontaneous recurrent seizures. Diazepam, phenobarbital, valproic acid and trimethadione protect against pilocarpine-induced status epilepticus while phenytoin and carbamazepine are ineffective. In the chronic phase, carbamazepine and phenytoin were effective against spontaneous seizures. Valproic acid was also effective against spontaneous seizures at the dose of 600 mg/kg and ethosuximide was ineffective against these seizures (Turski et al., 1987; Leite and Cavalheiro, 1995).

In the kainate model, ketogenic diet seems to be effective on reducing spontaneous recurrent seizures. Ketogenic diet-fed rats had significantly fewer and briefer spontaneous seizures and less supragranular mossy fiber sprouting, although the degree of hippocampal pyramidal cell damage was similar in both groups. (Muller-Schwarze et al., 1999).

6. Coupling neuroethology and EEG to evaluate behavioral seizure sequences

Intractable complex partial seizures rarely generalize even when drug doses are tapered during video-EEG monitoring. Nevertheless these oligosymptomatic seizures are often associated with lack of responsivity and amnesia to the seizure event and consequently have a considerable morbidity (Kotagal, 1991). Although in experimental epilepsy models it is possible to see analogous behavioral alterations, most of them are, because of their subtlety, frequently overlooked.
Fig. 1

A  TIME  CODE

00  FORELIMB MYOCLONUS
03  REARING
04  FALLING
05  FORELIMB MYOCLONUS
06  FALLING
07  OROFACIAL AUTOMATISMS

B

OROFA

C

WALKING
SNIFFING
LYING
AWAKE

BEHAVIORAL ARREST

OROFA

D

FREQUENCY
>100
65 - 100
27 - 64
0 - 26

X² Log
0,25-0,42
0,43-0,60
0,61-0,75
>0,75

DURATION
0-15
16-50
51-75
75-100

FORELIMB MYOCLONUS
TRUNK MYOCLONUS

F  Hippocampus

Amygdala

1 sec  2 mV
One of the reasons is that secondary generalized motor seizures, rather than partial ones, are easily detected by video monitoring setups. Although it is possible to couple motion detectors to video monitoring, class 4–5 limbic seizures are preferentially used as markers of epileptogenicity, in contrast with, for example, hippocampal and amygdala EEG seizure activity. For instance, a drug that suppresses a secondarily generalized seizure does not necessarily prevent episodes that would be an equivalent to a complex partial seizure in humans.

For this purpose, not only a high-resolution video capturing system is needed, but also a reliable system of behavioral sequence reconstruction. The method we will present here is based on quantitative ethology, first applied to epilepsy studies by Garcia-Cairasco and coworkers studying acute and kindled audiogenic seizures (Garcia-Cairasco et al., 1992, 1993, 1996). When behavior, captured in videos is analyzed, this approach detects statistically significant interactions ($P < 0.05; \chi^2$-test) of behavioral pairs (dyads) that are displayed in flowcharts (see Fig. 1). Then, depending of the type of behavioral sequence that is detected, some rules for the involved circuitry can be suggested.

The initial recording of the behavior, using a regular video camera and a video capturing board in order to digitalize the images, gives us time-behavior pairs such as those presented in Fig. 1A. The consequent behavioral sequence can be depicted as the flowchart shown in Fig. 1B, where rectangles represent specific behaviors and arrows represent links between behaviors. In Fig. 1A and B, the behavioral sequence of a spontaneous seizure induced by systemic application of pilocarpine is represented. Observe in this case a complex pattern of oro-facial automatisms, fore-limb myoclonus, rearing and falling. Furthermore, in order to get a dynamic picture of what happens with several animals, it is possible to display the average of a number of observation windows where spontaneous seizures are captured. In the current case, for each time window we used as behavioral trigger the presence of class six limbic seizures (Pinel and Rovner, 1978). In Fig. 1C is illustrated the resultant flowchart of nine spontaneous seizures observed after status epilepticus induced by systemic pilocarpine (380 mg/kg). In Fig. 1D, the calibration values of frequency and duration of behaviors are illustrated, as well as the statistical values, that link each behavioral pair in the flowcharts. In Fig. 1E and F are shown typical video-EEG screens of a partial seizure with recordings in the hippocampus and amygdala similar to Furtado et al. (2002).

Even secondarily generalized motor seizures can be easily detected with this ethologically based method and compared with the sequences of partial seizures shown in Fig. 1. Therefore, a comprehensive and coherent picture of both partial and generalized seizures can be evaluated, when specific observation times are chosen, for example, after drug treatments or when looking for age related effects. The observation time windows will depend on the experimental protocol.

The rational implicit in the current ethological evaluation is that the expression of brain activity, seen in linked motor acts, is highly correlated with behavioral sequences (Fentress and Stilwell, 1973; Norton, 1977; Garcia-Cairasco et al., 1992, 1996). Thus, behavioral events are recorded by conven-
tional video cameras and digitized by means of video capturing boards. After reviewing the sequences, times and behaviors are collected and processed in specifically developed software (Etho-
matic) that allows the reconstruction of, not only the sequence, but the statistical strength of behavioral interactions (Garcia-Cairasco et al., 1992). The flowcharts, which are the graphic expression of these sequences, are calibrated for behavior frequency, duration and statistical values. When evaluating either GABA and glutamate related drugs in experimental tonic-clonic seizures, neuroethological tools have proven to be more appropriate than seizure conventional scales, because flowchart analysis reveals behavioral associations that seizure severity scores usually do not detect (Terra and Garcia-Cairasco, 1992, 1994). Thus, for AED studies in pilocarpine and kainate models, sequential analysis will enable to build precise and reliable correlations between pharmacological effects on seizure behavior and involved brain substrates.

Analogous behavioral approaches to those described here are ethological studies after kainic acid evoked seizures in animals and studies based on cluster and flowcharts analysis in temporal and frontal lobe epileptic patients (Engel et al., 1991; Wieser 1991; Kotagal et al., 1995; Manford et al., 1996).

7. Conclusions

We conclude that pilocarpine and kainic acid are models that are useful for drug testing. In the status epilepticus phase, several drugs have proved to be effective. There is general agreement in clinical practice that prolonged seizures require aggressive therapy and that current AEDs are effective. Prolonged seizures can be terminated in most cases with intravenous administration of benzodiazepines, phenytoin, barbiturates and other compounds (Shinnar and Berg, 1996). Therefore, the need for new drugs does not seem to be of high priority. Studies on the latent phase seem to be more critical since there is a good chance that drugs or molecules that can block damage-induced plastic changes may also suppress the development of epilepsy. Studies from pilocarpine and kainate models have pointed out that AEDs such as valproate or other non-convulsant compounds such as basic fibroblast growth factor exert an antiepileptogenic effect (Liu and Holmes, 1997). The analogy of this latent phase with the acquisition of amygdala kindling should encourage testing of drugs that have proved to suppress the evolution of amygdala kindling (Cavalheiro et al., 1991; Silver et al., 1991). Levetiracetam and other effective compounds might be strong candidates (Loscher et al., 1998). Drug testing in the chronic phase should not address only the suppression of secondarily generalized motor seizures. Most of current tools used to quantify spontaneous seizure events need to be coupled to electrophysiology and more sophisticated systems for recording and analyzing behavior, such as the neuroethological approach discussed here.

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References


Baran, H., Loscher, W., Mevissen, M., 1994. The glycine/ NMDA receptor partial agonist α-cycloserine blocks kai-


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Sofia, R.D., Gordon, R., Gels, M., Diamantis, W., 1993. Effects of felbamate and other anticonvulsant drugs in...