The pilocarpine model of epilepsy

Cavalheiro E.A.
Neurologia Experimental, Escola Paulista de Medicina, São Paulo, Brasil

The systemic administration of a potent muscarinic agonist pilocarpine in rats promotes sequential behavioural and electrographic changes that can be divided in three distinct periods: (a) an acute period that built up progressively into a limbic status epilepticus and that lasts 24 h, (b) a silent period with a progressive normalization of EEG and behaviour which varies from 4 to 44 days, and (c) a chronic period with spontaneous recurrent seizures (SRSs). The main features of the SRSs observed during the long-term period resemble those of human complex partial seizures and recurs 2-3 times per week per animal. Therefore, this novel and unique experimental approach may serve as a model of epilepsy mimicking the human condition.

Key Words: pilocarpine — status epilepticus — limbic epilepsy, rats.

Introduction

The *Pilocarpine Model of Epilepsy* (PME) reproduces the main features of human temporal lobe epilepsy in rats and mice. The first evidence that rats with brain damage induced by pilocarpine-triggered status epilepticus could develop spontaneous recurrent seizures (SRSs) in the long run occurred in 1983 [15] when most of our work was devoted to the acute effects of the pilocarpine treatment. PME was fully characterized in rats in the following years [1, 5].

A single high dose of pilocarpine (300-380 mg/kg, ip), a potent muscarinic agonist originally isolated from the leaflets of South America shrubs, acutely induces sequential behavioural and electrographic changes indicative of sustained epileptic activity, resulting in widespread damage to the forebrain in both rats and mice [16, 17]. The initial alterations comprise akinesia, ataxic lurching, facial automatisms and head tremor. After 15-25 min those changes evolve to motor limbic seizures with rearing, forelimb clonus, salivation, intense masticatory movements and fall. Such fits recur every 2-8 min and lead to status epilepticus within 50-60 min after PILO administration that may last for up to 12 hours, rendering the animals prostrate or critically ill. The first 24 h taken by those manifestations, when the lethality rate reaches 30%, has been called ACUTE PERIOD.

Electrographic changes immediately after pilocarpine injection includes a significant theta rhythm superseding the background activity in the hippocampus (HPC) and low voltage fast activity in the cortex (CTX). This progresses to high voltage fast activity with spikes in the HPC. Sequentially, spiking activity spreads to the CTX and evolves into electrographic seizures, that recur every 2-8 min and finally become continuous within 50-60 min after pilocarpine administration. This pattern of electrographic activity is sustained for 6-12 hours and then gradually abates during the next 10-24 hours. Morphological analysis of the brains by the end of this period shows a characteristic damage preferentially distributed to the hippocampus (Fig. 1), thalamus, amygdaloid complex, pyriform and entorhynal cortex, neocortex and substantia nigra [11, 16]. The ultrastructural cell damage is that characteristically seen in protracted seizures [3], and includes massive swelling of dendrites and neuronal cell bodies (or vacuolar condensation), with axons being relatively spared, and dilatation of astroglial elements [3, 11].

After a "SILENT PERIOD" (seizure-free phase) that varies from 4 to 44 days (mean 14.8 ± 3.0 days) all surviving animals start exhibiting the

This research was supported by FAPESP, CNPq and FINEP from Brazil.

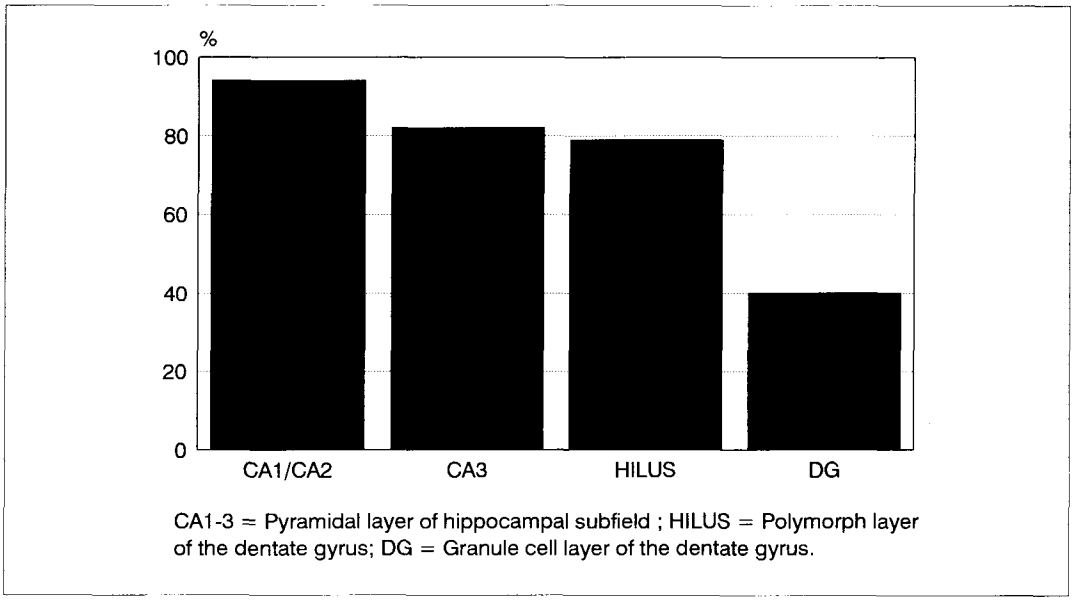

Fig. 1. *Percent of neuronal death in the hippocampal formation of rats following PILO-induced status epilepticus.*

SRSs which characterize the CHRONIC PERIOD, varying from 2 to 15 seizure episodes per month (mean of 2-3 seizures per week). Behaviourally, a spontaneous seizure is characterized by facial automatisms, head nodding, forelimb clonus, rearing and fall, and electrographically by paroxysmal hippocampal discharges that rapidly spread to cortical regions [1, 5]. No spontaneous remission of SRSs has been observed for as long as 6-month survival. The build-up of spontanous seizure activity that takes place from a few days after pilocarpine-induced SE match the behavioural and electrographic stages of kindling. The initial spontanous epileptic manifestation is usually characterized by paroxysmal hippocampal discharges with no changes in cortical records. Behavioural correlates of such electrographic features comprise an arrest reaction followed by eye blinking, chewing and head nodding, as in kindling stages 1 or 2 [12]. The following SRSs shows gradual electrographic synchronization of cortical and hippocampal activities and longer duration of ictal events (Fig. 2). Forelimb clonus, and rearing with falling (kindling stages 4 or 5) are the hallmarks of this phase. However, the duration of paroxymal discharges and sequence of behavioural stages varies widely in the spontaneous kindling that follows PILO-induced SE. Before the final stage 5 of kindling is reached, several convulsive stages of lesser intensity can be irregularly observed. Once the final stage is reached, essentially all the following seizures are also generalized. These observations demonstrate that the progression to fully developed SRSs seen in the PME is a kindling-related phenomenon [1. 5], with (1) primary activation of the hippocampus (previously to cortex), as observed in classical kindling, (2) unstable progression of kindling stages until secondary generalization, and (3) irregular duration of discharges before and after secondary generalization [14].

The validity of an experimental model of epilepsy is usually challenged by the response to known antiepileptic drugs [8]. Accordingly, studies on the efficacy of antiepileptic drugs against SRSs following pilocarpine-induced SE in rats would provide the necessary information about the validity this experimental model. To reach this goal, five clinically utilized antiepileptic drugs, phenobarbital (PHB), carbamazepine (CBZ), phenytoin (PHT), valproic acid (VPA) and ethosuximide (ESM), were administered after the establishment of the baseline seizure frequency for each rat. Seizure frequency during the two weeks of treatment was compared with equivalent periods before and after treatment. PHB (40 mg/kg), PHT (100 mg/kg) and CBZ (129 mg/kg) given daily were effective against spontaneous recurrent seizures. VPA, in the dose of 450 mg/kg was only weakly active, while in the dose of 600 mg/kg it was able to avoid the occurrence of SRS. ESM (400 mg/kg) was completely ineffective against SRSs during the two weeks of treatment [6]. The results obtained with these antiepileptic drugs

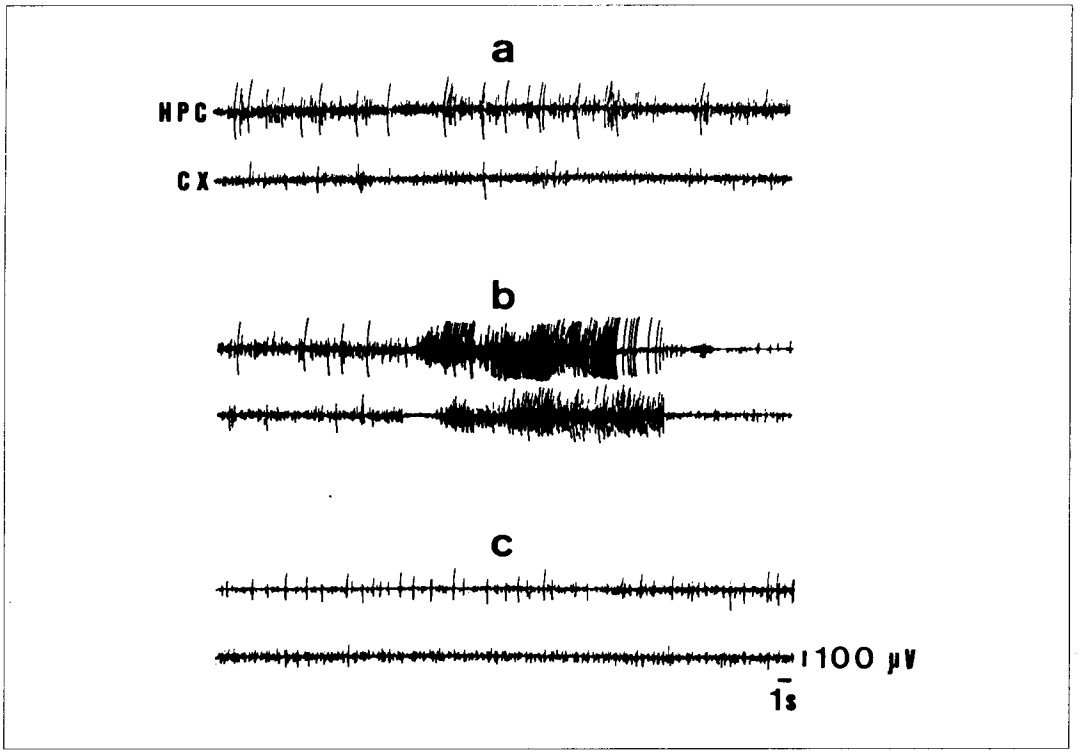

Fig. 2. *Electrographic recordings illustrating the buildup of a spontaneous recurrent seizure activity in an epileptic rat. The basal activity in the hippocampus (HPC) and cortex (CX) with isolated spikes is replaced by a burst of high-voltage beginning in the HPC and spreading to CX. a, b and c are continuous recordings.*

parallel their ability to protect against human complex partial seizures [4]. The similar profile of responses to antiepileptic drugs, together with the reproduction of electrographic, behavioural and neuropathological features of the clinical disorder, validate SRSs as a useful model of temporal lobe epilepsy.

As mentioned before, pilocarpine-induced SE invariably lead to cell loss in the hippocampal subfields CA1 and CA3 and in the hilus of the dentate gyrus. Cell loss is also often seen in the septum, olfactory tubercle, amygdala, piriform cortex, neocortex and thalamic nuclei [16, 18]. Timm staining of the hippocampal formation has shown supragranular sprouting of the mossy fibers which increases with time after the epileptogenic insult. Sprouting starts as early as 4 days after SE and reaches a plateau by 100-day survival [9]. The time course for the development of supragranular mossy fiber sprouting in PME is similar to that seen after epileptogenic or non-epileptogenic lesions of the hippocampal complex [2, 10, 13]. These results suggest that cell loss within the dentate hilus is a critical step that activates a cascade of events leading to mossy fiber sprouting, de novo recurrent excitation of granule cells, culminating in SRSs. In PME, as in clinical epilepsy, the silent period presumably represents an interval of active synaptic reorganization and sprouting, during which an initial insult produces a permanent disorder of the brain. Accordingly, during this interval therapeutic interventions may halt or redirect synaptic reorganization so as to prevent epileptogenesis. One interesting question regarding SE and secondary epileptogenesis refers to how sustained should be the acute epileptic activity for establishing a late irritative focus. To approach this problem we investigated the relationships among the duration of pilocarpine-induced SE, the latency for the appearance of the first SRS, the frequency of such seizures, the cell density in the hippocampal formation and the intensity of the reactive synaptogenesis observed through the Timm staining. An association of diazepam plus pentobarbital was used to interrupt SE at 30 minutes, 1, 2 or 6 hours after its beginning. SE remitted spontaneously in some non-treated rats. Animals submitted to 30 minutes of

SE did not evolve to the chronic period of PME up to 120-day recovery. A significant increase in the mean latency for the first spontaneous seizure and a significant decrease in SRS frequency were observed in animals following 1 and 2 hour SE. The behavioural and electrographic evolution of animals submitted to 6 hours of SE was indistinguishable from that observed in rats with spontaneous remission of SE. Cell loss in the hippocampal formation as well as the intensity of Timm staining varied proportionally with the duration of status epilepticus induced by pilocarpine [7]. These results show that the development of late epilepsy is closely related to the duration of status epilepticus. This relationship seems to be dependent on neuronal cell loss in the hippocampal formation and the subsequent synaptic reorganization in this structure.

Conclusion

The series of experiments reported here show that behavioural, electrographic, neuropathological and pharmacological response profiles of SRSs resemble some of the main features of human partial epilepsy [1, 6, 7, 9]. Therefore, this novel and unique model should be regarded as reliable experimental approach to study the human epileptic disorder. Furthermore, the evolution of SRSs indicates that several neurochemical changes induced by massive activation of hippocampal circuitry during SE leads to cell death and synaptic reorganization, that may underlie the development of the epileptic focus. Therapeutic intervention controlling the duration of SE or during the seizure silent period can modulate those alterations and prevent the late occurrence of SRSs.

Sommario

La somministrazione sistemica nei ratti di pilocarpina, un potente agonista muscarinico, determina modificazioni sequenziali sul piano comportamentale ed elettrografico che possono essere divisi in tre periodi distinti: a) un periodo acuto che determina progressivamente uno stato epilettico a livello limbico che dura 24 h; b) un periodo silente con una progressiva normalizzazione dell'EEG e dello stato comportamentale che varia da 4 a 44 giorni; c) un periodo cronico caratterizzato da crisi spontanee ricorrenti (SRSs). Le principali caratteristiche dell'SRSs osservate sul lungo periodo sono simili a quelle che si riscontrano nelle crisi parziali complesse umane e ricorrono in ogni animale con una frequenza di 2-3 volte alla settimana. Pertanto, questo nuovo ed unico approccio sperimentale può essere utilizzato come modello di epilessia che mima la condizione patologica umana.

References

[1] CAVALHEIRO E.A., LEITE J.P., BORTOLOTTO Z.A., TURSKI W.A., IKONOMIDOU C., TURSKI L.: *Long-term effects of pilocarpine in rats: Structural damage of the brain triggers kindling and spontaneous recurrent seizures.* Epilepsia 32:778-782, 1991.

[2] CAVAZOS J.E., GOLARAI G., SUTULA T.P.: *Mossy fiber synaptic reorganization induced by kindling: Time course of development, progression and permanence.* J. Neurosci. 11:2795:2803, 1991.

[3] CLIFFORD D.B., OLNEY J.W., MANIOTIS A., COLLINS R.C., ZORUMSKI C.F.: *The functional anatomy and pathology of lithium-pilocarpine and high dose pilocarpine seizures.* Neuroscience 23:953-968, 1987.

[4] EADIE M.J.: *Anticonvulsant therapy: Present and future.* TIPS 2:37-39, 1981.

[5] LEITE J.P., BORTOLOTTO Z.A., CAVALHEIRO, E.A.: *Spontaneous recurrent seizures in rats: An experimental model of partial epilepsy.* Neurosci. Biobehav. Rev. 14:511-517, 1990.

[6] LEITE J.P., CAVALHEIRO E.A.: *Antiepileptic drugs and their antagonism of spontaneous seizures in rats.* Epilepsia 32 (suppl. 1):35, 1991.

[7] LEMOS T., CAVALHEIRO E.A.: *Reorganização sináptica e ocorrência de crises espontâneas em ratos.* J. Liga Bras. Epil. 5:135-138, 1991.

[8] MELDRUM B.C.: *Preclinical test systems for evaluation of novel compounds.* In: B.S. Meldrum, R.J. Porter (eds.) New anticonvulsant drugs. London: John Libbey, pp. 31-48, 1986.

[9] MELLO L.E.A.M., CAVALHEIRO E.A., TAN A.M., KUPFER W.R., PRETORIUS J.K., BABB T.L., FINCH D.M.: *Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: Cell loss and mossy fiber sprouting.* Epilepsia, 39:985-995, 1993.

[10] NADLER J.V., PERRY B.W., COTMAN C.W.: *Selective reinnervation of hippocampal area CA1 and the fascia dentata after destruction of CA3-CA4 afferents with kainic acid.* Brain Res. 182:1-9, 1980.

[11] OLNEY J.W.: *Inciting excitotoxic cytocide among central neurons.* In: Y. Ben-Ari, R. Schwarcz. (eds.) Excitatory Amino Acids and Seizure Disorders. New York: Plenum Press, pp. 631-645, 1986.

[12] RACINE R.J.: *Modification of seizure activity by electrical stimulation. II. Motor seizure.* EEG Clin. Neurophysiol. 32:281-284, 1972.

[13] STEWARD O., VINSANT S.L., DAVIS L.: *The process of reinnervation in the dentate gyrus of adult rats: An ultrastructural study of changes in pre-*

synaptic terminals as a result of sprouting. J. Comp. Neurol. 267:24-33, 1988.

[14] TURSKI L., CAVALHEIRO E.A.: *Seizures induced by pilocarpine: Relevance in epilepsy.* In: N.N. Osborne (ed.) Current Aspects of the Neurosciences. New York: The Macmillan Press, pp. 127-158, 1992.

[15] TURSKI W.A., CZUCZWAR S.J., CAVALHEIRO E.A., TURSKI L., KLEINROK Z.: *Acute and long-term effects of systemic pilocarpine in rats: spontaneous recurrent seizures as a possible model of temporal lobe epilepsy.* Naunyn-Schmiedeberg's Arch. Pharmacol., 324:25R, 1983.

[16] TURSKI W.A., CAVALHEIRO E.A., SCHWARZ M., CZUCZWAR S.J., KLEINROK Z., TURSKI L.: *Limbic seizures produced by pilocarpine in rats: Behavioural, electroencephalographic and neuropathological study.* Behav. Brain Res. 9:315-335, 1983.

[17] TURSKI W.A., CAVALHEIRO E.A., BORTOLOTTO Z.A., MELLO L.E.A.M., SCHWARZ M., TURSKI L.: *Seizures produced by pilocarpine in mice: a behavioral, electroencephalographic and morphological analysis.* Brains Res. 321, 237-253, 1984.

[18] TURSKI L., CAVALHEIRO E.A., SIEKLUCKA-DZIUBA M., IKONIMIDOU-TURSKI C., CZUCZWAR S.J., TURSKI W.A.: *Seizures produced by pilocarpine: neuropathological sequelae and activity of glutamate decarboxylase in the rat forebrain.* Brain Res. 398:37-48, 1986.

Address reprint requests to: Esper A. Cavalheiro, Neurologia Experimental, Escola Paulista de Medicina, Rua Botucatu, 862 - 04023-900 São Paulo, SP, Brasil.