CONTRIBUTION OF IMAGING TECHNIQUES TO THE UNDERSTANDING OF THE PATHOGENESIS OF TEMPORAL LOBE EPILEPSY

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INTRODUCTION

Retrospective studies report patients with mesial temporal lobe epilepsy (MTLE) have a history of an initial precipitating injury (complicated febrile seizures, status epilepticus, encephalitis, head trauma, etc...). After a latency period of several years, development of recognizable MTLE begins towards the end of the first decade and commonly respond to appropriate antiepileptic drug treatment. Patients often become refractory to medication later in life and need surgery. In humans, MTLE is usually characterized by the unilateral lesion of the hippocampus, also called hippocampal sclerosis. Neuronal death often extends also to the ipsilateral amygdala, thalamus and temporal cortex (1, 2).

The model of MTLE induced by lithium-pilocarpine is well recognized and reproduces most clinical, developmental, and neuropathological features of human MTLE (3, 4). The initial precipitating injury is lithium-pilocarpine-induced status epilepticus (SE). In adult rats, the acute phase of SE is followed by a latent seizure-free phase of a mean duration of 14-25 days. During the latter phase, neuronal death develops bilaterally in hippocampus, amygdala, thalamus, septum, piriform and entorhinal cortex, neocortex, olfactory nuclei and substantia nigra. The large extent of neuronal death leads to circuit reorganizations that are causally linked the occurrence of spontaneous recurrent seizures (SRSs) in 100% of the animals (3-6), 2001; In addition, the consequences of lithium-pilocarpine SE are age-dependent (6-8). In rats aged less than 12 days (P12), lithium-pilocarpine SE does not lead to neuronal death or SRSs. (8) In P21 rats, SE-induced damage is less extended and pronounced than in adults and only 70-80% of the animals will develop epilepsy after a mean latency of 74 days (5, 6, 9).

To better understand the sequence of events leading to epilepsy and to identify the nature of the structures involved at the different phases of the epileptogenic process, and to define surrogate markers for epileptogenesis, in the present paper we will review our data on the lithium-pilocarpine model of epilepsy in the rat. We explored the events occurring at different phases of the epileptogenic process by using non invasive anatomic magnetic resonance imaging (MRI) to perform a temporal follow-up of the neuropathologic events occurring after SE, (11) [14C]2-deoxyglucose (2DG) quantitative autoradiography to measure rates of cerebral glucose utilization, (12) [14C]α-aminoisobutyric acid quantitative autoradiography to assess blood-brain barrier (BBB) permeability, (7) neuroprotection strategies to identify critical structures, (3) histology to quantify neuronal damage, and (9) video and EEG recording to assess the occurrence of SRSs. SE was induced...
by the injection of 3 mEq/kg of lithium chloride followed 18-20 h later by the injection of pilocarpine, 25, 30 and 60 mg/kg in adult, P21 and P10 rats, respectively.

MRI studies in adult and P21 rats

MRI was performed on a scanner operating at 4.7 T (SMIS). The whole brain was scanned on consecutive 1-mm thick coronal slices with a T2-weighted, spin-echo fast imaging method sequence (3800/80) to detect lesions. An additional spin-echo Carr-Purcell-Meiboom-Gill sequence (2500/22/8 echos) was performed to measure the T2 relaxation time.

In adult rats, MRI images showed the fast appearance of a hypersignal in piriform and entorhinal cortices as soon as 24 h after the onset of SE. This signal disappeared by 72 h and reflected the very fast neuronal damage in these areas which was totally achieved by 24 h. Conversely, in the hippocampus, the MRI signal appeared only by 30 h after SE but increased progressively with the evolution of the disease.

This temporal evolution reflected delayed neuronal death compared to the cortices and gliosis, both leading to hippocampal sclerosis. In the piriform and entorhinal cortical, the signal reappeared once the animals were epileptic, reflecting gliosis (10).

In P21 rats, all animals did not develop the same MRI signals. In one subgroup, no signal was found in the basal cortices; this group corresponds to the animals that will not become epileptic. In a second subgroup, as in adults, a signal appeared on MRI images in piriform and entorhinal cortices by 24 h after the onset of SE. These rats will all develop SRSs. Finally, in a third subgroup, although there was no hypersignal in the cortices on the T2 images, the quantification of T2 values showed a moderate transient increase at 24 h. These animals will also become epileptic (11).

Thus, it appears that piriform and entorhinal cortices are involved very early in the epileptogenic process and that their involvement is predictive of the epilepsy in P21 rats. As a confirmation of this observation, the measurements of local cerebral

![Fig.1: Temporal evolution of the T2-weighted images of brain sections from a single rat at different phases of the epileptogenesis induced by lithium-pilocarpine SE. At 24 h, there is a strong signal in basal cortices still present at 2 days when the hippocampal signal appears. These early signals reflect SE-induced necrosis. The cortical signal disappears by 72 h and reappears with the epilepsy reflecting gliosis. The hippocampal signal constantly increases in intensity over the whole process of epileptogenesis reflecting hippocampal sclerosis characterized by neuronal death and gliosis.](146)
glucose utilization by the 2DG technique showed in P21 and adult rats that did become epileptic, piriform and entorhinal cortices were the most activated structures during SE; while this did not occur in P10 rats that did not develop epilepsy, these structures were barely activated (Fernandes et al., 1999).

**BBB permeability in adult and P21 rats**

Prolonged convulsive activity produces an increase in cerebral capillary permeability and impairs BBB function in man (13) and many experimental models (14-15). BBB breakdown is transient and occurs early during epileptic activity (16). To identify the origin of the neuronal death induced by lithium-pilocarpine SE, we measured BBB permeability in adult and P21 rats using \[^{14}\text{C} \] \text{a-aminoisobutyric acid}, an aminoacid which does not cross an intact BBB, at 90 min after the onset of SE. BBB permeability increased both in structures that will undergo cell death (thalamus, septum, amygdala) and structures that will not be injured (globus pallidus, hypothalamus). Neuronal death occurred also in the absence of increased BBB permeability in hippocampus, entorhinal cortex and substantia nigra. The intensity of BBB permeability changes was more moderate in P21 than in adult rats (17).

Thus, there is no clear correlation between the anatomical distribution of BBB openings and the occurrence of neuronal death which, in this model, appears to rather depend on excitotoxic mechanisms due to major neuronal hyperexcitability.

**Neuroprotection strategies and epileptogenesis**

To determine the role of the different brain regions in the process of epileptogenesis, we applied both conventional and non conventional neuroprotection strategies.

The non conventional neuroprotection strategies consisted of preconditioning of the brain by the application of brief repeated seizures that do not result in neuronal death and lead to plastic changes at various levels of cell components (receptors, ion channels), reactivity (immediate early genes) and synthetic mechanisms (growth factors). We chose to compare the effects of amygdala kindling which involves mostly the forebrain limbic regions that will be later activated by lithium-pilocarpine SE, and of auricular maximal electroshocks (MES) that preferentially involve brainstem areas. A series of 10-11 fully kindled seizures or MES were applied prior to SE which was induced 24 h after the last brief seizure. We explored the effects of this preconditioning on neuronal damage and occurrence of SRSs.

Amygdala kindling prior to SE prevented the occurrence of neuronal death, totally the piriform and dorsal entorhinal cortex, amygdala, hippocampal CA1 and CA3 pyramidal cell layers, partially in ventral entorhinal cortex but does not protect the hilus. Chronic epilepsy developed in all animals after a similar latency whether the rats were kindled or not prior to SE. MES applied prior to SE did not provide any neuroprotection in the forebrain areas. Conversely, it worsened the neuronal death in entorhinal and perirhinal cortices. While all rats not subjected to MES prior to SE developed epilepsy after a mean latency of 40 days, only 2 rats that underwent MES became epileptic after a latency of at least 105 days (10).

The conventional strategies used drugs that were all applied after pilocarpine to prevent their direct effect on SE and discriminate their neuroprotective effect. Caffeine in drinking water or the injection of vigabatrin or topiramate led to the partial or total protection of CA1 and/or CA3 and vigabatrin and caffeine worsened the cell death in the entorhinal and piriform cortex, respectively (11, 18, 19). None of these treatments protected the hilus or influenced the latency before the first SRS. Pregabalin did not protect any hippocampal area but protected the piriform and entorhinal cortices. This treatment delayed the occurrence of the first SRS from 22 days in the control group to 39 days in the treated group (p<0.01) (12). Finally, the use of drug, not marketed yet allowed to protect CA1, CA3, the piriform and entorhinal cortices but not the hilus. The latency to the first SRS was increased by a factor of 8 (unpublished data) in some animals injected with this drug.

Thus, the sole protection of CA1 and CA3 is unable to prevent the development of epilepsy.
The protection of the basal cortices, entorhinal and piriform cortices contributes critically to delay the epileptogenesis: this effect is more pronounced when Ammon's horn is also protected. The hilus of the dentate gyrus is never protected and further strategies should aim at the protection of this key structure regulating the excitability of the hippocampal-entorhinal loop.

**Measurement of local cerebral glucose utilization during the latent and chronic phase**

Local cerebral metabolic rates for glucose (LCMRgls) were measured in rats subjected to lithium-pilocarpine SE at P10, P21 and as adults at the end of the latent phase (14 and 60 days after SE in adult and P21 rats, respectively) and during the interictal period of the chronic phase. Regional neuronal counts were performed to assess the relation between the extent of metabolic changes and the intensity of neuronal death. Indeed this relation remains controversial in human MTLE.

During the latent phase, in rats that underwent SE during adulthood or at P21, there was a good correlation between the extent of hypometabolism and neuronal drop out at both ages in most structures. Importantly, at both ages, although the intensity of neuronal death was high in the hilus of the dentate gyrus, LCMRGlc was in the normal range. The same situation was also observed in the piriform cortex of adult rats. These data suggest the remaining neurons in these structures were strongly activated when compared to the remaining neurons in some other structures in which neuronal death also occurs. During the interictal period of the chronic phase, the situation was similar to that recorded during the latent phase with the same relative hypermetabolism in the hilus of epileptic P21 and adult rats and in the piriform cortex of epileptic adult rats. In P10 rats studied during the latent and chronic phase, metabolic levels were in the normal range.

Thus, the metabolic studies confirm the prominent role of the hilus of the dentate gyrus (possibly enhanced by the piriform cortex in adult rats) in the initiation and maintenance of spontaneous epileptic seizures in the lithium-pilocarpine model.

**CONCLUSION**

Using the well recognized lithium-pilocarpine model of epilepsy, we demonstrated that the early activation of the basal cortices, mainly piriform and entorhinal, play a prominent and critical role during the first 24 h of the epileptogenic process. Later, during the latent and chronic phase, the hilus of the dentate gyrus, with the contribution of the piriform cortex in the mature brain, represents the critical structure for the initiation and maintenance of the spontaneous seizures which characterize the disease.

The present data confirm that imaging techniques are useful to understand the pathogenesis of a progressive disease such as MTLE. These techniques may help to define the critical steps in the disease. Hopefully, they will also help pediatric neurologists to identify early enough the children at risk to develop MTLE, and to intervene before the pathological circuit has been built and before the brain becomes epileptic, hence constantly functioning abnormally, both during and between the seizures.

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