Kainic acid induced hippocampal seizures in rats: comparisons of acute and chronic seizures using intrahippocampal versus systemic injections

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Hyppocampal epilepsy is a recently defined syndrome occurring in 65% of all temporal lobe epilepsies as defined by: 1) electrographic (EEG) onset in the hippocampus (HC) prior to EEG seizures elsewhere, 2) post-resection hippocampal sclerosis and mossy fiber synaptic reorganizations and 3) relief of typical complex partial seizures after surgical resection of the hyppocampus. We used intrahippocampal kainic acid injections V^2 in rats at different developmental ages (postnatal 7 through adult) to develop long term spontaneous HC EEG spikes, EEG seizures, and behavioral seizures. Split-screen video/EEG monitoring demonstrated that this intrahippocampal kainic acid model produced progressive development of: 1) ipsilateral interictal spikes, 2) later polyspike complexes, 3) bilaterally-asynchronous EEG spiking, 4) unilateral HC EEG seizure onsets with occasional secondarily generalized spread to apposite HC and motor cortex to elicit complex partial seizures, and 5) in all seizing rats there was mossy fiber synaptic reorganization, even when injected at age 7 days. These results indicate that the intrahippocampal kainic acid injection model is similar to human hippocampal epilepsy.

Key Words: temporal lobe epilepsy — partial seizures — synaptic reorganization — developing seizures — EEG/video.

In order to develop a rat model of human temporal lobe epilepsy, kainic acid (KA) was used to produce hippocampal cell loss and mossy fiber excitatory synaptic reorganizations [18]. These pathologic findings are constant findings in surgically-treated human hippocampal epilepsy [2, 4].

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Previous rat studies given intraperitoneal KA showed that after several weeks there was an association between fascia dentata mossy fiber sprouting and "hyperexcitability", as demonstrated by multiple spikes in granule cells in vitro [22] or by observed behavioral seizures [7]. Cavalheiro et al. [6] found spontaneous seizures 15 days after intrahippocampal KA, but recordings of EEG and behavior were discontinued after 50 days. Rats given intrahippocampal kainate have also shown sparing of fascia dentata GABAergic neurons and

sprouting of GAD containing axons into the inner molecular layer [8, 9] similar to human studies [3]. Mossy fiber sprouting in human hippocampal epilepsy extends to regio superior [4]. Similarly, Mathern et al. [15] showed that aberrant mossy fibers in the fascia dentata and regio superior progressively increased with time following intrahippocampal KA injury. After a physiologic latent period, both electrographic and partial complex behavioral seizures occurred, which is again similar to human hippocampal epilepsy [16, 17]. These spontaneous EEG and behavioral seizures were induced in adult rats given 0.2 μg/0.1 μl KA bilaterally and evolved, with an intervening latent period, only after 4 to 6 months post-KA. The present rat study used 0.4 μg/0.2 μl KA in one hippocampus and monitored for the acute, active, latent, and chronic phases described by Mathern et al. [16], with the addition of split-screen video/EEG and computer detection of regional spike densities and seizures. Intrahippocampal KA injections in adult rats (PN 30) were compared in parallel time course experiments to KA injections at PN 7, PN 10, PN 12, PN 14, PN 16, PN 18, and PN 21. As another comparison, intraperitoneal KA injections (10 mg/kg) were studied acutely. For review of KA acute effects, see Sperk [21]. In general, the disadvantages to systemic KA, (intraperitoneal, subcutaneous, intravenous) and to a certain extent intraventricular injections, include: 1) the variability in calibrating the relatively small amount of kainate available to cause hippocampal damage [5], 2) the damage to hippocampus depends on age, weight and strain [12], and 3) the damage is widespread (e.g. pyriform, entorhinal and other neocortical regions, [19]). At present, there is no evidence to demonstrate that following systemic KA long-term spontaneous seizures specifically originate from the hippocampus similar to human partial complex focal hippocampal or temporal lobe seizures. Hence, the present research strategy was to ensure focal hippocampal damage at different ages and attempt to define critical periods for damage and progressive epileptogenesis.

Methods

Seventy male Sprague-Dawley rats ages postnatal 7 days (PN 7) through adult (PN 30, PN 50), were used and included ages PN 10, PN 12, PN 14, PN 16, PN 18, PN 21. KA (0.4 μg/0.2 μl) was stereotactically-injected over a twenty-minute period into the right occipital-bend of the temporal hippocampus [Anterior to Lambda: 2.1 mm (PN 7) to 2.5 (PN 30); Lateral to midline: 3.6 (PN 7) to 4.5 (PN 30); vertical from cranium 4.0 (PN /) to 5.0 (PN 30)], according to the Atlas of Sherwood and Timiras, 1970. A control normal saline vehicle of 0.2 μl was similarly injected into the homologous left hippocampus. Bipolar recording electrodes were chronically-implanted bilaterally in the hippocampi (HC) and frontal cortices (FC), according to Mathern et al. [16]. Both bipolar and chain-linked EEG montage recordings were performed, digitized into a computerized EEG system, and formatted with a video signal to produce a split-screen EEG and video image. The computerized EEG was monitored using on-line spike and seizure detection programs, and EEG epochs were stored on hard disk and then transferred to an optical disk for reformatting of EEG montages. The localization and quantification of EEG spike densities in relation to the right KA injection site were determined from short (2 months) through long-term (6 months) periods.

TABLE I. *Spike densities (sample epoch 41 or 99 hrs) 140 days post-KA (PN 30).*

	FC	Left HC	Right HC
Rat 12 (41 hrs.):	0 spikes/hr	22.2 spikes/hrs.	111.8 spikes/hr.
Rat 6 (99 hrs.):	3.9 spikes/hr.	7.0 spikes/hr.	47.5 spikes/hr

The relation of all these short and long-term EEG changes from rats given intrahippocampal KA at different postnatal ages were related to hippocampal cell loss in the hilus (cresylviolet stain) and mossy fiber (MF) synaptic reorganization (neo-Timm histochemistry: 0.1% w/v Na_2S) in the supragranular fascia dentata (FD), according to previously described techniques [16].

Results and Conclusions

I. *Acute* intrahippocampal KA seizures in adult rats consisted of *ipsilateral* to the KA injection EEG spikes, long after discharges, and partial limbic status epilepticus (with mastication and wet-dog shakes). In rat pups, (PN 7 through PN 14) *acute behavior* was characterized by turning, scratching, and falling, associated with shaking which was more prominent in older rats (e.g. PN 16, 18). No EEG recordings were taken during the acute seizures in such small rats. However, short-term hippocampal recordings have been described following *intraperitoneal* KA in PN 15 rats [1, 13] and neither EEG activity nor behavior was localized to the HC.

II. *Long-term spike densities*: At 5 months post-KA, interictal EEG and behavior was very similar to that reported for medically-intractable, surgically-treatable temporal lobe epilepsy in man, (for

Fig. 1. *Split-screen photomontage of a spontaneous seizure 5 months after intrahippocampal KA in PN 30 rat. The EEG is characterized by a hypersynchronous, high-voltage spiking (bottom recording). Second, third and fourth pictures depict the sequence of behavioral changes during the rearing phase. Note the blurring effect caused by the rapid bilateral clonic jerks of forelimbs. The seizure ends abruptly and is characterized by a depressed background EEG activity (fifth and sixth pictures).*

reviews see [10, 11, 14]. Although the rat has strong hippocampal commissures compared to human, most computer-detected interictal spikes were focal and localized to the right (KA) hippocampus, with less EEG spiking in left HC and rarely in FC (see Table 1).

III. Long-term (5 months post-KA) spontaneous seizures were very similar to complex partial seizures associated with hippocampal sclerosis in man in that rats exhibited initial immobility, star-

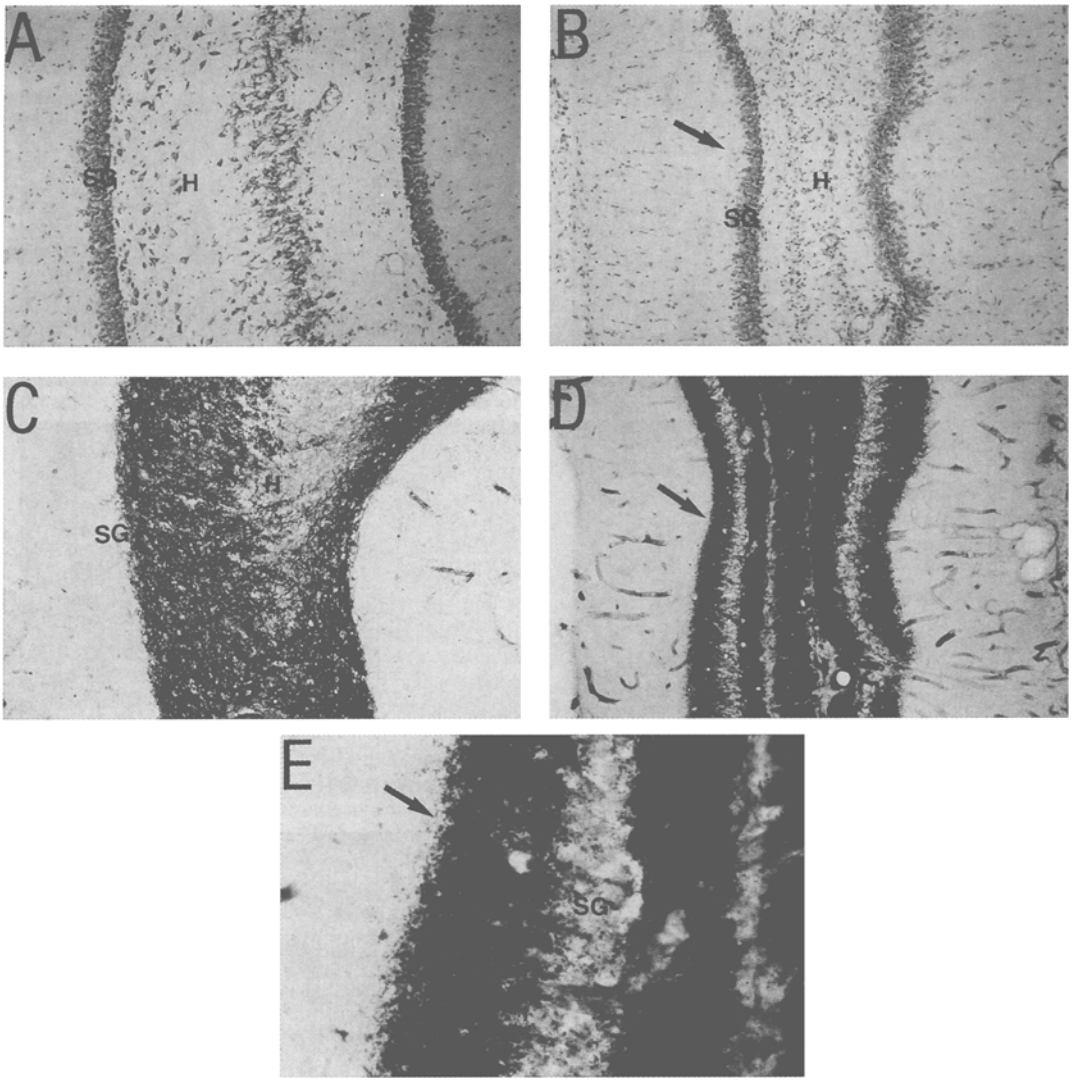

Fig. 2. *Nissl (A,B) and Timm (C,D,E) stained sections of hippocampus from the same animal shown in Fig. 1. A. Nissl-stained section showing normal neuron density of the hilar region (H) in the saline-injected side. B. KA-injected side showing extensive neuronal loss and collapse of the hilus with relatively preserved granule cells (SG). C. Neo-Timm stained section of the saline-injected side showing the characteristic, normal mossy fiber hilar staining, with no Timm puncta in the supragranular layer. D. Section adjacent to B stained with neo-Timm showing extensive supragranular mossy fiber sprouting (arrow). E. Higher magnification of the same region indicated by the arrow in D. Note the dense mossy fiber terminal punctae in the stratum granulosum (SG) and inner molecular layer. These pathology findings are reliably found in adult human hippocampal epilepsy.*

ing, eventually mastication, facial movements, forelimb movements, and finally generalization of EEG associated with rearing and rapid, alternating forelimb clonus. The most severe seizures could last over 3 minutes and were found in rats given KA either as a pup (PN 7) or as an adult (PN 30) Figure 1 is a split-screen photomontage of the typical EEG and behavioral stages of one such seizure, which occurred 5 months after KA intrahippocampal injection in a PN 30 rat. Note the rearing and rapid forelimb clonus alternating from left to right paw, which lasted over 20 sec. Figure 2 shows the histopathology (hilar atrophy) and the dense molecular layer mossy fiber synaptic reorganization in the supragranular region of the FD, which is an identical pattern constantly

found in human hippocampal sclerosis.
Our studies to date have shown that *all* rats that exhibited either very intense or mild hippocampal seizures also had such supragranular MF sprouting. However, there were rats that exhibited supragranular MF sprouting without necessarily exhibiting severe behavioral seizures but did show interictal spikes. Hence, "sprouted" hippocampi *always* generated EEG spikes and polyspike complexes, often showed HC EEG after discharges, and in some cases showed subclinical or partial seizures. Since the rats were not *all* monitored 24 hours daily, the incidence of EEG and clinical seizures cannot be fully documented. However, we can conclude that in the absence of MF supragranular sprouting, there were few HC EEG abnormalities; rather the HC EEG often appeared to have normal adult theta rhythms especially in the PN 7 through PN 14 KA rats. Obviously, the present techniques need more study; however the findings provide an excellent rat model that replicates the anatomic pathology and neurophysiology of human temporal lobe (hippocampal) epilepsy.

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Sommario

L'epilessia ippocampale è una sindrome recentemente definita che si riscontra nel 65% di tutte le epilessie del lobo temporale ed è caratterizzata da: 1) esordio elettrografico (EEG) nell'ippocampo (HC) precedente l'attività EEG critica nelle altre strutture; 2) riscontro di sclerosi ippocampale e di riorganizzazione sinaptica delle fibre muscoidi nel tessuto asportato chirurgicamente; 3) regressione delle tipiche crisi parziali complesse in seguito a resezione chirurgica. Noi abbiamo iniezioni di acido cainico in ratti di diversa età postnatale (da P7 all'età adulta) per ottenere punte EEG persistenti in HC, attività EEG critica e crisi comportamentali. Il monitoraggio video-EEG ha dimostrato che in questo modello ottenuto con iniezione intraippocampale di acido cainico si osserva il progressivo sviluppo di: 1) punte EEG interittali ipsilaterali; 2) in uno stadio successivo complessi di polipunta; 3) scariche di punte EEG bilaterali asincrone; 4) crisi parziali complesse associate a scariche EEG che iniziano nell'HC di un lato e si propagano occasionalmente all'HC del lobo opposto e alla corteccia motoria; 5) riorganizzazione sinaptica delle fibre muscoidi in tutti i ratti con crisi anche se iniettati precocemente (P7).
Questi risultati indicano che il modello ottenuto con iniezione intraippocampale di acido cainico è assimilabile all'epilessia ippocampale umana.

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