EVOLUTION OF HIPPOCAMPAL EPILEPTIC ACTIVITY DURING THE DEVELOPMENT OF HIPPOCAMPAL SCLEROSIS IN A MOUSE MODEL OF TEMPORAL LOBE EPILEPSY

V. Riban, a,b V. Bouilleret, c B. T. Pham-Lê, a J.-M. Fritschy, b C. Marescaux a and A. DEPAULIS a

aNeurobiologie et Neuropharmacologie des Epilepsies Généralisées, INSERM U. 398, Faculté de Médecine, 11 rue Humann, F-67085 Strasbourg Cedex, France
bInstitute of Pharmacology and Toxicology, Winterturstrasse 190, CH-8057 Zurich, Switzerland

Abstract—Unilateral intrahippocampal injection of kainic acid in adult mice reproduces most of the morphological characteristics of hippocampal sclerosis (neuronal loss, gliosis, reorganization of neurotransmitter receptors, mossy fiber sprouting, granule cell dispersion) observed in patients with temporal lobe epilepsy. Whereas some neuronal loss is observed immediately after the initial status epilepticus induced by kainate treatment, most reorganization processes develop progressively over a period of several weeks. The aim of this study was to characterize the evolution of seizure activity in this model and to assess its pharmacological reactivity to classical antiepileptic drugs.

Intrahippocampal electroencephalographic recordings showed three distinct phases of paroxysmal activity following unilateral injection of kainic acid (1 nmol in 50 nl) into the dorsal hippocampus of adult mice: (i) a non-convulsive status epilepticus, (ii) a latent phase lasting approximately 2 weeks, during which no organized activity was recorded, and (iii) a phase of chronic seizure activity with recurrent hippocampal paroxysmal discharges characterized by high amplitude sharp wave onset. These recurrent seizures were first seen about 2 weeks post-injection. They were limited to the injected area and were not observed in the cerebral cortex, contralateral hippocampus or ipsilateral amygdala. Secondary propagation to the contralateral hippocampus and to the cerebral cortex was rare. In addition hippocampal paroxysmal discharges were not responsive to acute carbamazepine, phenytoin, or valproate treatment, but could be suppressed by diazepam.

Our data further validate intrahippocampal injection of kainate in mice as a model of temporal lobe epilepsy and suggest that synaptic reorganization in the lesioned hippocampus is necessary for the development of organized recurrent seizures. © 2002 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: animal model, kainic acid, hippocampus, focal seizures, electroencephalographic recordings, epileptogenesis.

Partial complex seizures with hippocampal sclerosis (HS) represent one of the most common forms of medically refractory epilepsies. These seizures are usually of focal type and are associated with typical emotional reactions and motor automatisms (Engel, 1996b). Occasionally, they can secondarily propagate to limbic structures and to the cerebral cortex, leading to a generalized seizure. Histopathologically, temporal lobe epilepsy (TLE) associated with HS is characterized by neuronal death, most severe in the CA1 region and in the hilus of the dentate gyrus, and reactive gliosis (for review, see Margerison and Corsellis, 1966; Gloor, 1991; Sloviter, 1994). In about 50% of patients, a dispersion and hypertrophy of the dentate gyrus granule cells are also observed (Houser, 1990; Lurton et al., 1998; Loup et al., 2000). The etiology and pathogenesis of these histological changes are disputed (Lurton et al., 1997). However, TLE with HS is often preceded by an initial precipitating event in early childhood. A latent period is observed between this initial episode and the first epileptic seizure, during which alterations leading to epileptogenesis may occur.

It has been shown recently that unilateral injection of kainic acid (KA) into the dorsal hippocampus of adult mice induces a pattern of cell loss and synaptic reorganization reminiscent of the alterations observed in TLE with HS (Suzuki et al., 1995; Bouilleret et al., 1999). In addition, a prominent enlargement of the dentate gyrus constitutes a unique feature of this model (Suzuki et al., 1995), which has not been reported in rat models of TLE. While the morphological changes in the hippocampal formation have been extensively investigated and have further validated this model of HS (Bouilleret et al.,
2000b,c), only superficial electroencephalographic (EEG) recordings were performed. These recordings revealed recurrent paroxysmal activity from the first day on, with no changes in behavioral manifestation, for up to 1 year after KA injection (Bouilleret et al., 1999). On the basis of these superficial EEG recordings, it was not possible to distinguish between ictal and interictal activities or to assess the occurrence of a latent period. In addition, there has been no pharmacological analysis of the effect of antiepileptic drugs in this model.

The aim of this study was to characterize in detail the evolution of paroxysmal activity initiated by injection of KA in this model, using intrahippocampal EEG recordings. We have focused on three points: (1) the evolution of paroxysmal activity after the initial lesion to determine the presence or not of a latent period, (2) the localization of the paroxysmal activity in relation to the lesion site, (3) the pharmacological responsiveness of this model to classical antiepileptic drugs.

**EXPERIMENTAL PROCEDURES**

**Animals and surgery**

Experiments were conducted on 64 Swiss male mice (35-40 g) (Rj:Sw, Janvier, le-Genest-St-Isle, France) housed in individual cages with food and water ad libitum and kept in a 12-h light-dark cycle. All animal procedures were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize animal suffering and reduce the number of animals used.

Two groups of 30 mice were stereotaxically injected under general anesthesia (Equithesin, 4 ml/kg i.p.) with 50 nl of a 20 mM solution of KA in 0.9% NaCl (i.e., 1 mmol) into the right dorsal hippocampus (anteroposterior (AP)=1.8, mediolateral (ML)=+1.8, dorsoventral (DV)=-1.9 mm) with bregma as reference using a stainless steel cannula (outer diameter, 0.28 mm) connected to a 0.5-μl microsyringe (Hamilton, Bonaduz, Switzerland) via PE20 tubing filled with distilled water. Each injection was performed over 1 min using a micro-pump (CMA/100, Carnegie Medicin, Stockholm, Sweden). At the end of the injection, the cannula was left in place for an additional 1-min period to limit reflux along the cannula track. In addition, four control mice were injected with saline under the same conditions.

After intrahippocampal injection, all mice were implanted with three monopolar surface electrodes placed over the left and right frontoparietal cortex and over the cerebellum (reference electrode) and with a bipolar electrode inserted into the injected hippocampus. The monopolar electrodes were made of a tungsten wire (diameter, 250 μm) soldered on a male connector (Wire pro, Farnell, France). They were inserted in the skull so that only the tip (0.5 mm) protruded onto cortical or cerebellar tissue. The bipolar electrode was formed of two twisted enamel insulated stainless steel wires (diameter, 170 μm, distance between the tips, 0.4 mm) connected to a male connector. It was aimed at the right hippocampus with the same coordinates as for the injection site. In four mice, the implantation was performed 2 months after KA injection to verify that chronically implanted electrodes do not influence the development of seizures. Finally, three KA-injected mice were implanted with an additional bipolar electrode in the contralateral hippocampus (AP=+1.8, ML=1.8, DV=-1.9) and four others with a monopolar electrode in the ipsilateral amygdala (AP=-1.7, ML=-3.1, DV=-4.7). Electrodes were fixed to the skull with cyanoacrylate and dental acrylic cement. The mice were then allowed to recover from anesthesia before being placed in the EEG recording chamber (see below).

**Electroencephalographic recordings**

EEG activities were recorded using a digital acquisition computer-based system (Coherence, Delhamed, France, max. 32 acquisition channels, sampling rate 200 Hz) in freely moving animals placed in a Faraday cage. One group of mice was used for characterization of the EEG at various time points after KA injection, the second group for pharmacological analysis (see next section). A referential setup was used in which cortical, hippocampal or amygdalar electrodes were referenced with an electrode placed over the cerebellum. This setup allowed visualizing EEG activity in different derivations after the acquisition. Status epilepticus was recorded for up to 10 h after the end of surgery in 20 mice. Each animal from the first group was then recorded at regular intervals (two or three times per week) for 2 months after the injection, totaling at least 20 sessions per mouse. Each EEG session was performed from 16.00 to 19.00 h, during the resting phase of the mice. They were first habituated to the test cage and then recorded for 2.5 h.

**Drug administration**

The effects of antiepileptic drugs on the frequency of recurrent seizures were assessed on a separate group of mice exhibiting typical hippocampal paroxysmal discharges at 2-3 weeks post-KA. Valproate (Sanofi Recherche, Paris, France) (n=6) and phenytoin (Parke-Davis, Paris, France) (n=5) were dissolved in saline on the day of experiment and injected i.p. in a volume of 1 ml/kg. The commercial form of diazepam (Roche, Paris, France) dissolved in propylene glycol (5 mg/ml) was injected in a volume of 0.5 ml/kg (n=6). Carbamazepine (Novartis, Basel, Switzerland) was first dissolved in Molecusol (2-hydroxypropyl-β-cyclodextrin, RBI, Natick, MA, USA) and then distilled water at a final concentration of 15 mg/ml (n=6). All pharmacological compounds were tested between the third and the sixth week following KA treatment. After a reference EEG recording period of 40 min, the animals received one of the different doses of the drug under study or solvent in a randomized order and were recorded for 80 min. Each animal was thus used as its own control, with a delay of at least 3 days between two injections.

**Histological controls**

Upon completion of the experiments, all mice were injected with a lethal dose of pentobarbital (Nembutal, 100 mg/kg, i.p.). Their brains were removed, frozen and cut into 20-μm sections using a cryostat. Histological analysis was performed following Cresyl Violet staining to verify (i) the location of the KA injection, (ii) the location of the hippocampal electrode, and (iii) the pattern of neuronal loss and the dispersion of dentate gyrus granule cells, as previously reported (Bouilleret et al., 1999; Knuesel et al., 2001). Eight animals were discarded because the lesion was too small (n=6) or the bipolar electrode misplaced and hippocampal EEG activity not clearly visible (n=2).

**Statistical analysis of drug effects**

The effects of antiepileptic drugs were assessed by counting the number of hippocampal paroxysmal discharges in a 40-min period post-injection. In view of the variability in the number of seizures between trial sessions, the number of seizures during the 40-min reference period was used as a covariance factor. For each compound, the number of recorded seizures during the 40-min period post-injection was compared between doses using a one-way analysis of variance with repeated measures. Post-hoc comparisons versus control conditions (injection of solvent) were performed using the Bonferroni test. Statistical significance was set at P<0.05.
RESULTS

Histological consequences of intrahippocampal kainic acid injection

Among the 60 mice injected with KA, 12 died during the first 3 weeks. Histological controls performed 2 months after the injection of KA in the remaining animals revealed in 42 cases a typical pattern of hippocampal damage on the injected side, characterized by extensive neuronal loss in the CA1 and CA3c regions and in the hilus of the dentate gyrus. In addition, an enlargement of the dentate gyrus with dispersion of the granule cells was observed in all these animals. These changes were restricted to the dorsal hippocampus and no modifications were observed contralaterally (Fig. 1), as described previously (Bouilleret et al., 1999; Knuesel et al., 2001). In saline-injected mice \((n=4)\), discrete gliosis was observed along the track of the hippocampal electrode. No signs of lesion were noted otherwise.

In most cases, it was not possible to determine the exact site of injection. However, in KA-treated mice that died during the first week after the injection, the trace of the cannula ended between the CA1 area and the upper blade of the dentate gyrus. In these animals, the tip of the hippocampal electrode was located in the upper blade of the dentate gyrus. Likewise, in mice killed after 2 months, the tip of the hippocampal electrode was located in the enlarged upper blade of the dentate gyrus. In six animals injected with KA, no pattern of cell loss and granular cell dispersion was observed. Since no signs of paroxystic activities were recorded in these animals, they were discarded from the study, along with two additional mice in which the bipolar electrode was misplaced and hippocampal EEG activity not clearly discernible.

Encephalographic and behavioral consequences of intrahippocampal injection of kainic acid

The effects of KA treatment were investigated by intrahippocampal EEG recordings during three distinct phases: status epilepticus, upon awakening from surgery \((n=20)\), latent phase, up to 2 weeks post-KA \((n=18)\); chronic phase, from 2 weeks to 2 months post-KA \((n=17)\). Mice injected with saline were taken as controls \((n=4)\).

Status epilepticus

After surgery, mice treated with KA and saline were continuously EEG recorded for up to 10 h. Upon awakening from anesthesia, hippocampal EEG recording of control mice (Fig. 2A) revealed either typical desynchronized activity or theta activity (5–7 Hz) during exploration. In contrast, the EEG activity in mice injected with KA was characterized by regular isolated spikes, spike-and-wave, and polyspikes (200–600 µV, 1–3 Hz) recorded concomitantly on both ipsilateral and contralateral hippocampal and cortical derivations during 6–10 h (Fig. 2B). No theta activity was observed and the amplitude of hippocampal background activity was lower bilaterally (50–100 µV) than in control animals (500–900 µV). In approximately half of the animals, mild clonic movements of the forelimbs and rotations were observed during this period, the other half remained immobile and prostrated. Generalized clonic seizures were observed in eight mice during this period. These were associated with concomitant high frequency spikes or polyspikes on the hippocampal and cortical derivations. These mice did not present any differences compared with the others in terms of EEG or histological pattern. After 2 days, all animals had recovered and no gross behavioral impairment was observed.

Chronic recordings

EEG recordings during the 2 months follow-up were performed during the resting phase of the mice, typically from 16.00 to 19.00 h. In mice injected with saline, EEG recordings showed a typical desynchronized activity in the hippocampus when the animals were awake. In addition, theta rhythm (5–7 Hz) was often observed on the hippocampal derivation concomitantly with active sniffing, exploration and sleep, as previously described. Occa-

![Fig. 1. Morphological alterations induced by unilateral KA injection into the dorsal hippocampus, as seen in transverse sections stained with Cresyl Violet at 3 weeks post-injection. (A) Contralateral, non-injected hippocampus, displaying the normal histology of the hippocampal formation. (B) Ipsilateral, KA-treated side. Neuronal loss is evident in the hilus of the dentate gyrus (DG), as well as in CA1 and parts of CA3. A pronounced dispersion of dentate gyrus granule cells is also visible. Scale bar = 200 µm.](NSC 5516 22-5-02)
sionally, isolated low voltage spikes (800–1100 µV) could be observed. Likewise, no paroxystic activity was recorded in the cerebral cortex of control mice.

In all KA-treated mice analyzed, ipsilateral hippocampal EEG recording during the 2 months follow-up showed a complete disappearance of the theta rhythm, which was replaced by a low voltage background activity (150–300 µV) (Fig. 3A). In contrast, background activity in the contralateral hippocampus was normal and could not be distinguished from control mice. In the KA-injected hippocampus, the following four electroencephalographic patterns were recorded.

**Low voltage spikes** (Fig. 3A): From the end of the status epilepticus and only during the first 2 weeks, low voltage spikes or spike-and-waves (600–900 µV, 100–150 ms) were recorded in the injected hippocampus and simultaneously on the cerebral cortex in 90% of the animals. These spikes occurred sporadically and were sometimes grouped in short discharges (3–4 Hz, 1–3 s). No noticeable behavior was observed during the occurrence

---

**Fig. 3. EEG recordings during the first 2 weeks (latent period) after intrahippocampal KA injection.** Upper trace: ipsilateral intrahippocampal derivation; lower trace: ipsilateral cortical derivation. (A) Background activity (left part of the trace) and low voltage spikes occurring either isolated or grouped in sequence. The boxed area is enlarged on the right to show that spikes occur concomitantly in the cortex, which may be due to volume conduction. Low voltage spikes were recorded daily in the hippocampus during the first 2 weeks. (B) Bursts of high frequency activity (arrows), which could be recorded only during one session at the end of the latent phase.
of these spikes. After the first 2 weeks following KA injection, these spikes were never observed again.

**Bursts** (Fig. 3B): Between 13 and 15 days after KA injection, bursts of high frequency and low voltage spikes (300–500 µV, 18–26 Hz) preceded by a single sharp wave (700–1500 µV, 200–300 ms) were recorded in the ipsilateral hippocampus in some animals. This activity could only be recorded during 1 or 2 days in the same animal and was never observed in another period after KA injection.

**High voltage sharp waves** (Fig. 4A): Following these transient high frequency bursts, high voltage sharp waves (1500–4500 µV, 150–200 ms) were recorded in all mice. They occurred either isolated or grouped in bursts (3–6 Hz, 4–10 s). In mice implanted bilaterally with bipolar electrodes, such sharp waves occurred with some delay (8–12 ms) in the contralateral hippocampus, but no activity was observed on the cortical derivations. No concomitant behavior was noticed. These activities first appeared 2 weeks after KA injection and were then observed until the animals were killed.

**Hippocampal paroxysmal discharges** (Fig. 4B): Two weeks after KA injection, high voltage sharp waves as described above (1500–4500 µV, 3–5 Hz) followed by higher frequency and low voltage rhythmic activity (10–14 Hz, 700–1100 µV) were recorded in all animals. These hippocampal paroxysmal discharges lasted between 20 and 60 s and their rate of occurrence was variable between animals with a maximum of one discharge every other minute. The rate of occurrence of hippocampal paroxysmal discharges was also highly variable within the recordings of a given animal and sometimes no hippocampal paroxysmal discharges could be recorded for hours. These discharges were observed only in the ipsilateral hippocampus and not contralaterally or in cerebral cortex. Behavioral arrest with head nodding could be observed concomitantly with hippocampal paroxysmal discharges. However, some animals also displayed stereotyped behavior, such as exploration or grooming. In the four mice implanted with a bipolar electrode in the hippocampus 2 months after KA injection, hippocampal paroxysmal discharges...
as well as sharp waves were observed with a similar pattern as described in mice implanted on the day of injection.

When the tips of the electrode were located at the same anteroposterior level as the injection site, hippocampal paroxysmal discharges were recorded at either tip (derivations with the reference electrode). In two mice where the electrode was located at a different anteroposterior level (i.e., more than 0.5 mm distant from the injection site), theta rhythm could be recorded and no hippocampal paroxysmal discharges were observed.

Discharges propagating to the cerebral cortex (Fig. 5). In 19 of the 40 animals, low voltage fast activity observed during the hippocampal paroxysmal discharges was occasionally prolonged and spread successively to the ipsilateral cortex, the contralateral hippocampus and the contralateral cortex. Such discharges lasted about 60 s and were accompanied by clonic movements of the forelimbs occurring when the low voltage fast activity was propagating to the cortex. A flattening of EEG activity for 20–60 s always followed these seizures.

Fig. 5. EEG recordings of paroxystic activities during the chronic phase following intrahippocampal KA injection. Upper trace: ipsilateral intrahippocampal derivation; lower trace: ipsilateral cortical derivation. Example of paroxystic activity propagating to the cerebral cortex and leading to a generalized clonic seizure. This seizure is followed by a characteristic post-ictal depression.
**EEG recordings in the amygdala.** Four animals were implanted with a monopolar electrode in the ipsilateral amygdala in addition to the bipolar electrode in the hippocampus. One died 3 days after KA injection. Similar patterns of EEG activity as described above were observed at the hippocampal derivation. During status epilepticus, sporadic low voltage slow spikes (200–400 µV) were recorded in the amygdala. They appeared concomitantly with hippocampal spikes. During the first 2 weeks following injection, concomitant spikes were observed. Thereafter, no paroxystic activity was recorded any more in the amygdala concomitantly with hippocampal paroxysmal discharges or sharp waves in the hippocampus.

**Effects of antiepileptic drugs**

The effects of acute injections of four antiepileptic drugs classically prescribed to patients with TLE were tested in different groups of animals between 3 and 6 weeks after the injection of KA (Fig. 6). Mice were selected for these experiments based on EEG recordings at 15–18 days post-KA showing the typical paroxystic activities described above. The doses of antiepileptic drugs were adapted from the literature.

Following injection of **diazepam** (2.5 mg/kg, i.p., n = 6), hippocampal paroxysmal discharges and sharp waves were suppressed for at least 40 min, as compared to vehicle injection. No clear sedative effect was observed at this dose.

Following injection of the low dose of **valproate** (40 mg/kg, i.p., n = 6), a non-significant increase of hippocampal paroxysmal discharge occurrence was observed as compared to vehicle injection. Higher doses of valproate (100, 140 mg/kg, i.p., n = 6, respectively) did not result in any significant difference in the number of hippocampal paroxysmal discharges as compared to saline injection. No modification of sharp waves was noticed, although no quantitative analysis was performed. Three mice were tested with a dose of 200 mg/kg at the end of the experiment, but no noticeable modifications of either the background EEG activity or the behavior of these animals were observed.

Injection of **phenytoin** (25, 50 mg/kg, i.p., n = 5) did not result in any significant difference in the number of hippocampal paroxysmal discharges as compared to saline injection. In addition, phenytoin at these doses did not modify the sharp wave pattern and no behavioral modifications were noticed. Injection of 100 mg/kg was followed in all mice by a profound disturbance of the hippocampal EEG, with a prolonged high frequency and low voltage activity in the discharge and occurrence of tonic–clonic seizures 30 min after injection. Motor disturbances lasting for up to 2 days were also observed.

Injection of **carbamazepine** (30 mg/kg, i.p., n = 6) did not result in any significant modifications of the number of hippocampal paroxysmal discharges and did not induce any pattern alterations, as compared to vehicle injection. No modifications of the sharp wave pattern were observed. No higher dose could be tested due to the low solubility of this compound.

**DISCUSSION**

This study shows that intrahippocampal injection of KA in adult mice results in profound, progressive and long-term modifications of hippocampal EEG activity. Using intracerebral recordings, three different phases can be distinguished in this model: (i) a non-convulsive status epilepticus initiated immediately after KA injection and persisting for up to 10 h, (ii) a latent phase, lasting for about 2 weeks and characterized by sporadic low voltage spikes or spike-and-waves, and (iii) a chronic phase characterized by recurrent high voltage sharp waves and hippocampal paroxysmal discharges.

These spontaneous discharges are limited to the lesioned hippocampus and are suppressed by diazepam but not by other classical antiepileptic drugs. Our data further confirm that this model of hippocampal sclerosis is reminiscent of TLE with HS.

**Intrahippocampal injection of kainic acid in mice induces focal epileptic activity after a latent phase**

The main result of the present study is that alterations caused by unilateral intrahippocampal injection of KA in adult mice lead to the occurrence of two recurrent paroxystic patterns: (i) a rhythmic activity of high voltage sharp waves and (ii) hippocampal paroxysmal discharges, characterized by a sequence of high voltage sharp waves followed by a sequence of lower voltage and higher frequency spikes-and-waves associated with behavioral arrest or stereotypies. Both patterns were never observed in saline-injected animals and cannot therefore be due to mechanical lesions or the long-term presence of the depth electrode. In addition, similar patterns were observed in mice treated with KA but implanted only 2 months later. Moreover, both patterns were not observed during the acute phase after KA injection and are therefore not caused directly by the injection. Indeed, these EEG patterns appeared only 2 weeks after KA injection.
after the injection and could be recorded for more than 2 months without changes in their characteristics.

Our data thus demonstrate that spontaneous recurrent paroxysmal discharges in the hippocampus following local injections of KA appear after a latent period of at least 2 weeks. During this period, a profound change in the hippocampal activity is observed, with the disappearance of normal background activity and frequent occurrence of low voltage spikes. As suggested by our recordings in some animals, these spikes progressively occur as bursts following a single sharp wave. This brief period of bursting activity could be recorded around the 15th day post-injection. It was not observed in all of the animals, since they were recorded every 2–3 days. Whereas the underlying mechanisms remain to be explored, these bursts might represent a transition between the activity observed during the latent period and the onset of hippocampal paroxysmal discharges. Although more experiments are necessary to determine the changes in the activity of individual neurons, our study suggests that hyperactivity of hippocampal neurons induced by the loss of inhibitory interneurons (Bouilleret et al., 2000b) may progressively become synchronized and result in the occurrence of organized discharges beginning with sharp waves.

The three distinct phases observed in the present study (status epilepticus, latent phase, and chronic seizures) model the disease history of patients with TLE with HS (Engel, 1996a,b). Similarly, the existence of three phases has been described in most animal models with an initial induced status epilepticus, but very few data are available on hippocampal activity during the latent phase (Bragin et al., 2000). Our results demonstrate that this period certainly cannot be considered ‘silent’ in the mouse model of KA injection. Due to the focal nature of hippocampal paroxysmal discharges and sharp waves, previous superficial EEG recordings (Bouilleret et al., 1999) failed to distinguish between the latent and chronic phases. It was therefore an essential feature of the present study to obtain detailed recordings from the lesioned hippocampus.

**Intrahippocampal injection in mice induces epileptic activity limited to the injected hippocampus**

Using depth electrodes and a referential setup, it was possible in the present study to better characterize the recurrent paroxysmic patterns occurring after the latent period and persisting without noticeable modifications for up to 2 months. Single or grouped high voltage sharp waves were regularly recorded at the hippocampal electrode located near the injection site. They were sometimes observed on the contralateral hippocampus, but never in cerebral cortex. The amplitude and duration of these sharp waves suggest that they result from the synchronization of a large population of neurons. Such sharp waves have been described in other animal models (Buzsaki, 1986; Bragin et al., 1999) as well as in humans (Engel, 1996a; Velasco et al., 2000). In these studies, sharp waves were not limited to the epileptic focus, but could also be recorded in entorhinal cortex or in the contralateral hippocampus. They were not associated with any particular change in behavior, as in the present study. These sharp waves may be considered interictal events dissociated from seizures. However, when they occur as bursts, they may initiate hippocampal paroxysmal discharges. The relationship between these high voltage sharp waves and hippocampal paroxysmal discharges remains to be examined to determine their role in the generation of hippocampal seizures.

Contrary to the high voltage sharp waves that were observed sometimes in the contralateral hippocampus, hippocampal paroxysmal discharges were restricted to the injected hippocampus. They were associated with concomitant changes in behavior, which varied between animals. Either behavioral arrest with slight head nodding or stereotyped exploration or sniffing was observed. Clonic movements of the limbs only occurred in mice having a generalized seizure. Thus, hippocampal paroxysmal discharges share several features with seizures observed in patients with TLE using stereo EEG recordings. In these patients, two main ictal events have been described. One is characterized by low voltage, fast activity and is followed by a progressive increase of the amplitude and slowing of the rhythm. This pattern is observed when the discharge involves amygdala and other temporal regions and spreads more readily to the cortex (Park et al., 1996; Velasco et al., 2000). Such a pattern was not observed in mice in the present study. The second pattern, occurring in most patients, usually begins with hypersynchronous high voltage spikes limited on one derivation of the depth electrode (Engel, 1990; Velasco et al., 2000; Blume et al., 1984). This pattern is observed preferentially in sclerotic hippocampus and particularly when this structure is the focus of epileptic activity (Park et al., 1996). This pattern therefore shows strong similarities with hippocampal paroxysmal discharges recorded in the present study. In addition, hippocampal paroxysmal discharges appear limited to the epileptic focus, since they cannot be recorded when the electrode is placed outside the lesion area in the dorsal hippocampus.

Hypersynchronous or low voltage fast activity ictal onsets have also been described in the rat following intrahippocampal injection of KA (Bragin et al., 1999). According to this study, both patterns of onset can be recorded in the same animal suggesting that different propagation circuits are involved. A major difference between this rat model and mice injected with KA is the dispersion of the granule cell layer in the latter. It is therefore possible that this neuroplasticity phenomenon contributes to the stability of the epileptic circuit in mice. It is also conceivable that the pattern of ictal activity depends on the extent of neuronal loss within the hippocampus (Velasco et al., 2000) or on the localization of the recording electrode (Bragin et al., 1999). These hypotheses can be tested with further recordings at different electrode locations in the mouse model.

The fact that hippocampal paroxysmal discharges are limited to the vicinity of the lesioned area during the chronic period is in agreement with previous observations using magnetic resonance imaging in this model.
During this period, a hyper signal in T2-weighted images was limited to the lesioned area in the dorsal hippocampus (Bouilleret et al., 2000a). It is also in agreement with previous studies using different models showing a strong association between epileptic focus and sclerotic area (Babb et al., 1984). This focal aspect of hippocampal activity appears, however, to develop during the latent period. During this phase, EEG abnormalities were recorded in the amygdala, the cortex, and contralateral hippocampus in the present study. They were generally concomitant with hippocampal paroxysmal activity. Similarly, T2-weighted signals were observed in the amygdala during the latent period (Bouilleret et al., 2000a). This suggests that the loss of hippocampal projections may limit the spread of hippocampal paroxysmal discharges outside the injected area. It is also possible that inhibitory processes develop during the latent period that may stop the seizures before propagation.

**Pharmacological resistance**

The present study suggests that hippocampal paroxysmal discharges and interictal sharp waves in KA-treated mice are resistant to phenytoin, valproate, or carbamazepine, but suppressed by diazepam. The lack of effectiveness of classical antiepileptic drugs is in agreement with the pharmacological reactivity observed in patients with TLE. Benzodiazepines have been shown to be suppressive upon acute administration but lose their effect when given chronically. This loss of antiepileptic effect with daily administration of diazepam was also obtained within a few days in this model (personal observation).

For patients with medically refractory TLE with HS, surgical resection of the affected hippocampal formation represents the only therapeutic alternative. Although animal models have been widely used to screen antiepileptic drugs and to study their effect on hyperexcitable circuits, little is known about the basis of pharmacoresistance in TLE with HS.

Furthermore, in view of a clearly defined latent period, this model allows investigating early treatment strategies initiated after the first episode of injury and aiming to prevent morphological alterations and the development of chronic epilepsy. To date, there is no effective treatment protecting against epileptogenesis or delaying the onset of the first epileptic seizure. But it is widely recognized that the reorganization of hippocampal circuit taking place after the precipitating injury is likely to result in chronic recurrent seizures. Chronic studies are therefore planned on this model to study the potential effect of long-term antiepileptic drug treatment on epileptic network organization.

Altogether, these results suggest that the pharmacological reactivity of recurrent seizures induced by intrahippocampal injection of KA in mice shares similarities with pharmacoresistance observed in TLE patients. This study also demonstrates that this model represents a valuable tool to investigate the molecular and cellular basis of pharmacoresistance and to screen new potential antiepileptic drugs against focal seizures.

**Epileptogenesis**

The extensive morphological characterization of this model allows correlating changes in the cytoarchitecture in the lesioned hippocampus with patterns of seizure activity. It has been shown that the intrahippocampal injection of KA induces a rapid loss of most hilar cells, including mossy cells and interneurons. Furthermore, interneurons expressing parvalbumin and calbindin rapidly disappear in CA1 and dentate gyrus (Bouilleret et al., 2000b). Most of this cell loss was complete within 24 h post-injection, indicating that it is not sufficient to trigger per se an organized epileptic activity. The latent phase of 2 weeks therefore appears a critical period of reorganization of hippocampal circuits leading to the onset of recurrent seizures.

During this period, several morphological and functional modifications occur. In the dentate gyrus, granular cells show an increase in their body size and extraneuronal space, which doubles during the first 2 weeks and then progressively increases for several months (Suzuki et al., 1995). Extensive sprouting of mossy fiber terminals has been reported in the supragranular layer of the dentate gyrus and in the infrapyramidal blade of CA3 (Suzuki et al., 1995; Bouilleret et al., 2000b). Dentate gyrus granule cells also show de novo expression of neuropeptide Y, dynorphin and glutamic acid decarboxylase (GAD), suggesting that they acquire a dual excitatory/inhibitory phenotype (Makiura et al., 1999). In addition, although there is a gradual loss of type 1 GABA transporter immunoreactivity in granular cells, up-regulation of several GABA<sub>A</sub> receptor subunits has been reported in these neurons, suggesting functional plasticity of GABAergic signaling (Bouilleret et al., 2000b; Knuesel et al., 2001). Finally, reorganization of different α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor subunits in granule cells has been reported recently in this model (Suzuki et al., 2000). Similar neuroplasticity phenomena have been reported in studies using resection tissues from medial temporal lobe epilepsy patients (Mathern et al., 1995; Blumcke et al., 1996; Loup et al., 2000).

Although further studies are necessary to determine the functional consequences of the hippocampal reorganization observed during the first weeks following KA injection, the increased GABAergic function suggested by loss of transporter staining and the up-regulation of GABA<sub>A</sub> receptors might contribute to the hypersynchronization phenomena responsible for paroxysmatic activities. It should be noted, however, that most plasticity changes initiated during the first 2 weeks progress during several months thereafter. In particular, mossy fiber sprouting, up-regulation of GABA<sub>A</sub> receptors, and granule cell dispersion reach a maximum 2–3 months after the KA injection. In spite of this, no modifications in the pattern of rhythmic sharp wave activity and hippocampal paroxysmal discharges were observed during the chronic phase. The role of the different morphological and functional modifications of the dentate gyrus – as well as other parts of the hippocampus – in the establishment
and maintenance of these paroxystic activities remains to be determined.

CONCLUSION

The characterization of seizures affecting KA-treated mice indicates that this model replicates several key features of TLE with HS: (i) presence of a latent period following a precipitating injury, (ii) paroxysmal ictal activity during the chronic phase is limited to the lesion area, and (iii) paroxysmal ictal and interictal activity are resistant to classical antiepileptic drugs. Several morphological (dispersion, sprouting) and functional (expression of GAD, neuropeptide Y, somatostatin, up-regulation of GABA<sub>A</sub> and AMPA receptors) modifications of dentate gyrus granule cells have been described during the latent period, which may be responsible for the occurrence of recurrent seizures.

Intrahippocampal injection of KA in mice thus represents a valuable model to investigate the molecular and cellular basis of epileptogenesis in TLE associated with HS. This model may be used to test new potential antiepileptic compounds against focal seizures. Furthermore, in view of a clearly defined latent period, this model allows investigating early treatment strategies initiated after the first episode of injury and aiming to prevent morphological alterations and the development of chronic epilepsy.

REFERENCES


(Accepted 16 January 2002)