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Various modifications of the intrahippocampal kainate model of mesial temporal lobe epilepsy in rats fail to resolve the marked rat-to-mouse differences in type and frequency of spontaneous seizures in this model

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ABSTRACT

Temporal lobe epilepsy (TLE) is the most common type of acquired epilepsy in adults. TLE can develop after diverse brain insults, including traumatic brain injury, infections, stroke, or prolonged status epilepticus (SE). Post-SE rodent models of TLE are widely used to understand mechanisms of epileptogenesis and develop treatments for epilepsy prevention. In this respect, the intrahippocampal kainate model of TLE in mice is of interest, because highly frequent spontaneous electrographic seizures develop in the kainate focus, allowing evaluation of both anti-seizure and anti-epileptogenic effects of novel drugs with only short EEG recording periods, which is not possible in any other model of TLE, including the intrahippocampal kainate model in rats. In the present study, we investigated whether the marked mouse-to-rat difference in occurrence and frequency of spontaneous seizures is due to a species difference or to technical variables, such as anesthesia during kainate injection, kainate dose, or location of kainate injection and EEG electrode in the hippocampus. When, as in the mouse model, anesthesia was used during kainate injection, only few rats developed epilepsy, although severity or duration of SE was not affected by isoflurane. In contrast, most rats developed epilepsy when kainate was injected without anesthesia. However, frequent electrographic seizures as observed in mice did not occur in rats, irrespective of location of kainate injection (CA1, CA3) or EEG recording electrode (CA1, CA3, dentate gyrus) or dose of kainate injected. These data indicate marked phenotypic differences between mice and rats in this model. Further studies should explore the mechanisms underlying this species difference.

1. Introduction

Intrahippocampal injection of the neurotoxic glutamate analogue kainate has become a widely used model of inducing limbic status epilepticus (SE) in mice and rats that, after a seizure-free latent period, is followed by development of spontaneous recurrent seizures (SRS) reminiscent of mesial temporal lobe epilepsy (TLE) in patients [1–4]. This model has significantly contributed to the understanding of the molecular, cellular and neurophysiological mechanisms underlying ictogenesis and epileptogenesis [4]; however, use of the model in rats for testing of novel anti-seizure or anti-epileptogenic drugs is impeded by the low frequency of SRS [5]. In apparent contrast to rats, in mice the intrahippocampal kainate model is characterized by an extremely high frequency of spontaneous electrographic seizures recorded from the kainate focus in the ipsilateral hippocampus, allowing testing of the anti-seizure effect of novel drugs during the 1–2 h that follow their administration [3,6], which is not possible in any other model of TLE.

The availability of anti-epileptogenic or disease-modifying treatments for patients at risk for developing epilepsy after brain injury is a major unmet clinical need [7,8]. We recently proposed a two-stage preclinical approach for developing such treatments [9]. In stage 1, tolerability and efficacy of potential anti-epileptogenic drugs or drug combinations is tested in the intrahippocampal kainate mouse model of TLE as recently described for the AMPA receptor antagonist NBQX [10]. The reason for choosing the mouse model for stage 1 is the high frequency of spontaneous electrographic seizures which greatly facilitates anti-epileptogenic drug testing [9,10]. In stage 2, tolerable and effective compounds or combinations of compounds resulting from stage 1 are further evaluated in the intrahippocampal kainate model of TLE in rats, thus allowing determination of whether the effect observed in mice translates to another species [9]. For this two-stage approach it would be highly desirable to modify the rat model in a way that epileptic animals exhibit as frequent electrographic seizures as mice in the intrahippocampal kainate model of TLE.

Abbreviations: EEG, electroencephalogram; HPD, hippocampal paroxysmal discharge; HVSW, high-voltage sharp waves; SE, status epilepticus; SRS, spontaneous recurrent seizures; TLE, temporal lobe epilepsy.

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Thus, the main aim of the present study was to refine or modify the rat intrahippocampal kainate model, so that a similar high frequency of spontaneous electrographic or nonconvulsive seizures is obtained as in the mouse model. For this aim, we first had to analyze the major differences in the methods used in most studies on this model in rats and mice. As shown in Table 1, in most studies in rats, kainate was injected in awake (non-anesthetized) animals, whereas anesthesia is commonly used in mice. Furthermore, although the average brain of a rat, independent of sex, is at least 5-times larger than a mouse brain [11], the typical intrahippocampal doses of kainate used in rats and mice only vary by a factor of two. In addition, the localization of kainate injection in the hippocampus differs in rats and mice as does the localization of the depth electrode used for EEG recording (Table 1). We therefore studied in rats whether injecting kainate under anesthesia (as in mice), changing the intrahippocampal kainate injection site (as in mice), recording the EEG from the kainate focus (as in mice), or increasing dose and volume of the intrahippocampal kainate results in a model that more closely parallels the mouse model in terms of high-frequency electrographic seizures. To our surprise, most of these modifications of the rat model, particularly anesthesia during kainate injection, did not only fail to reproduce the advantages of the mouse model but instead resulted in less rats developing epilepsy.

2. Materials and methods

2.1. Animals

As in our previous studies in this model [5,12], we used female Sprague–Dawley rats, which were purchased at an age of 9 weeks (body weight 200–220 g) from either Janvier (Le Genest-Saint-Isle, France) or Harlan (Udine, Italy). Because rats from the two vendors did not differ in their response to intrahippocampal injection of kainate or subsequent development of SRS, they were grouped together for final analysis of data. Following arrival in our laboratory, the rats were kept under controlled environmental conditions (22–24 °C; 50–60% humidity; 12 h light/dark cycle; light on at 6:00 a.m.) with free access to standard laboratory chow (Altromin 1324 standard diet; Altromin, Lage, Germany) and tap water. Rats were maintained individually in transparent Makrolon polycarbonate type III cages (38 × 22 × 20 cm). Transfer to new cages was once per week. All rats were adapted to the laboratory and habituated to handling and injections for at least one week before starting the experiments. During the course of the experiments, the animals were handled two to three times per week; during this handling the rats remained about 1–2 h in an open field where they had social interaction with other rats. Age at time of SE induction was 11–13 weeks. Female rats were housed without males in order to keep them acyclic or asynchronous with respect to their estrous cycle [cf.,13,14]. In a preliminary study in the intrahippocampal kainate model, in which vaginal smears were taken in 21 rats to determine the stage of the estrous cycle at time of SE induction, no relationship between estrous cycle and SE severity or duration was observed [12].

Experiments were performed according to the EU council directive 2010/63/EU and the German Law on Animal Protection ("Tierschutzgesetz"). Ethical approval for the study was granted by an ethical committee (according to §15 of the Tierschutzgesetz) and the government agency (Lower Saxony State Office for Consumer Protection and Food Safety; LAVES) responsible for approval of animal experiments in Lower Saxony. All efforts were made to minimize both the suffering and the number of animals. The experimental protocol is illustrated in Fig. 1.

2.2. Implantation of EEG electrodes and guide cannulae for microinjection

Two groups of rats were compared: (1) rats in which kainate was injected under anesthesia with isoflurane (groups 1a–1e) and (2) rats in which kainate was injected without anesthesia (groups 2a–2c). In

Table 1
Typical experimental protocols and their outcome in the intrahippocampal kainate model of epilepsy in rats and mice. Abbreviations: SE, status epilepticus; SRS, spontaneous recurrent seizures.

Variable	Intrahippocampal kainate model in rats	Intrahippocampal kainate model in mice	References (representative examples)
Anesthesia during kainate injection	No (injection via guide cannula in conscious animals)	Yes (chloral hydrate, isoflurane or Equithesin [commercial mixture of chloral hydrate, magnesium sulfate, and pentobarbital sodium])	Bragin et al. [16]; Raedt et al. [34]; Riban et al. [19]; Gouder et al. [24]; Gröticke et al. [26]; Rattka et al. [5]; Töpfer et al. [42]; Twele et al. [10,53]; Klein et al. [6]
Dosage of kainate	0.4 μg (=1.9 nM)	0.21 μg (=1 nM)	As above
Localization of kainate injection	CA3 (dorsal hippocampus)	CA1 (dorsal hippocampus)	As above
Localization of EEG electrode	CA3, CA1 or dentate gyrus	CA1 (in kainate focus)	As above
Type of SE	Limbic interrupted by generalized convulsive seizures	Limbic interrupted by generalized convulsive seizures	As above
Percentage of animals developing SRS after SE	>80%	>80%	As above
Types of spontaneous convulsive seizures	Focal onset secondarily generalized convulsive seizures	Focal onset secondarily generalized convulsive seizures	As above
Types of electrographic seizures (recorded from the ipsilateral hippocampus)	None reported	High-voltage sharp waves (HVSWs; bursts of sharp waves with 3–8 Hz, an amplitude of 1.5–4.5 mV, and a duration of 4–20 s) and hippocampal paroxysmal discharges (HPDs; bursts of spikes and poly-spikes with 10–20 Hz, an amplitude of 0.5–1.1 mV, and a duration of 20–60 s). Both types only occur in the ipsilateral CA1 within 0.5 mm of the kainate injection site.	Bragin et al. [16]; Raedt et al. [34]; Riban et al. [19]; Maroso et al. [20]; Rattka et al. [5]; Töpfer et al. [51]; Klein et al. [6]; Twele et al. [10.53]
Frequency of spontaneous convulsive seizures	Irregular with large interindividual variation (on average 1–2 per week); progression to higher frequency later during epilepsy	Irregular with large interindividual variation (on average 1–2 per week)	Gouder et al. [24]; Bragin et al. [16]; Gröticke et al. [26]; Raedt et al. [34]; Riban et al. [19]; Maroso et al. [20]; Rattka et al. [5]; Töpfer et al. [51]; Klein et al. [6]
Frequency of electrographic seizures	None reported	HVSWs: 20–100 per h HPDs: 30–100 per h	Riban et al. [19]; Arabadzisz et al. [52]; Töpfer et al. [51]; Klein et al. [6]; Twele et al. [10,53]
Hippocampal pathology	Extensive neuronal loss in CA3 and dentate hilus, and dispersion of granule cell layer in ipsilateral hippocampus	Extensive neuronal loss in CA1, CA3c, and dentate hilus, and dispersion of granule cell layer in ipsilateral hippocampus	Bouilleret et al. [23]; Riban et al. [19]; Gouder et al. [24]; Gröticke et al. [26]; Raedt et al. [34]: Lévesque and Avoli [4]; Rattka et al. [5]