Epilepsy & Behavior 55 (2016) 47–56

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Significant effects of sex, strain, and anesthesia in the intrahippocampal kainate mouse model of mesial temporal lobe epilepsy

Friederike Twele, Kathrin Töllner, Claudia Brandt, Wolfgang Löscher *

Department of Pharmacology, Toxicology, and Pharmacy, University of Veterinary Medicine Hannover, Germany
Center for Systems Neuroscience, Hannover, Germany

ARTICLE INFO

Article history:
Received 23 October 2015
Revised 24 November 2015
Accepted 24 November 2015
Available online 28 December 2015

Keywords:
Seizures
Gender
Chloral hydrate
Isoflurane
Epileptogenesis
Latent period
Status epilepticus

ABSTRACT

The intrahippocampal kainate mouse model of mesial temporal lobe epilepsy is increasingly being used for studies on epileptogenesis and antiepileptogenesis. Almost all previous studies used male mice for this purpose, and no study is available in this or other models of acquired epilepsy that directly compared epileptogenesis in female and male rodents. Epidemiological studies suggest that gender may affect susceptibility to epilepsy and its prognosis; therefore, one goal of this study was to investigate whether sex has an influence on latent period and epileptogenesis in the intrahippocampal kainate model in mice. Another aspect that was examined in the present study was whether mouse strain differences in epileptogenesis exist. Finally, we examined the effects of different types of anesthesia (chloral hydrate, isoflurane) on kainate-induced status epilepticus (SE) and epileptogenesis. Continuous (24/7) video-EEG monitoring was used during SE and the 2 weeks following SE as well as 4–6 weeks after SE. In male NMRI mice with chloral hydrate anesthesia during kainate injection, SE was followed by a seizure-free latent period of 10–14 days if hippocampal paroxysmal discharges (HPDs) recorded from the kainate focus were considered the onset of epilepsy. Anesthesia with isoflurane led to a more rapid onset and higher severity of SE, and not all male NMRI mice exhibited a seizure-free latent period. Female NMRI mice differed from male animals in the lack of any clear latent period, independently of anesthesia type. Furthermore, HPDs were only rarely observed. These problems were not resolved by decreasing the dose of kainate or using other strains (C57BL/6, FVB/N) of female mice. The present data are the first to demonstrate marked sex-related differences in the latent period following brain injury in a rodent model of acquired epilepsy. Furthermore, our data demonstrate that the choice of anesthetic agent during kainate administration affects SE severity and as a consequence, the latent period, which may explain some of the differences reported for this model in the literature.

1. Introduction

About 40% of all epilepsies result from acute brain injuries including traumatic brain injury, ischemic stroke, intracranial hemorrhage, tumors, infections, and prolonged acute symptomatic seizures such as complex febrile seizures or status epilepticus (SE) [1]. The time between a brain injury and the onset of spontaneous recurrent seizures (SRS) is termed "latent period" [2–4]. During the latent period, there is a cascade of poorly understood changes, termed "epileptogenesis", that transform the nonepileptic brain into one that generates SRS [5]. The most common form of epilepsy developing after acute brain injuries is temporal lobe epilepsy (TLE), which is difficult to control by antiseizure drugs (ASDs) and is often associated with severe comorbidities that may, at least in part, be a consequence of neuronal damage, particularly in the hippocampus [6,7]. Despite an enormous number of studies on the brain alterations occurring during epileptogenesis and possible targets to interfere with these alterations, there are no clinically available antiepileptogenic drugs, which could prevent epilepsy when administered after an epileptogenic brain injury [8]. Thus, development of novel drugs that prevent or modify epilepsy in patients at risk is an urgent medical need [8]. For this purpose, animal models of epileptogenesis are an important tool [3,9]. During development of new antiepileptogenic treatments, gender-related aspects need to be dealt with [10–12].

Epidemiological studies suggest that gender may affect susceptibility to epilepsy and its prognosis; however, only limited research has been dedicated to the impact of gender on susceptibility to acquired epilepsies so that the effect of gender on susceptibility and clinical evolution of acquired epilepsies is largely unknown [13]. The same is true for preclinical research [12,14]. Most preclinical studies on epileptogenesis and targets for antiepileptogenesis have been performed in male rodents, and no

Abbreviations: ASD, antiseizure drug; HPD, hippocampal paroxysmal discharge; HVSW, high-voltage spike waves; LVS, low-voltage spikes; SE, status epilepticus; SRS, spontaneous recurrent seizures; TLE, temporal lobe epilepsy.

* Corresponding author at: Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine, Bünteweg 17, D-30559 Hannover, Germany. Tel.: +49 511 856 8721; fax: +49 511 953 8581.
E-mail address: wolfgang.loescher@tiho-hannover.de (W. Löscher).

http://dx.doi.org/10.1016/j.yebeh.2015.11.027
1525-5050/

study is available that directly compared epileptogenesis in female and male rodents [4,12]. There are various rodent models of acquired epilepsy [3], but most studies on epileptogenesis and antiepileptogenesis have been performed with post-SE models of TLE [4,12,15]. In such models, SE is induced either chemically or electrically; in chemical models, convulsants such as pilocarpine or kainate are either administered systemically or intracerebrally in rats or mice [3]. Scharfman and MacLusky [14] recently reported sex differences in SE induction by systemic administration of pilocarpine but not kainate in rats, but the influence of sex on development of epilepsy after SE was not examined.

In the present study, we used the intrahippocampal kainate model of mesial TLE to evaluate sex differences in epileptogenesis in mice. In this model, unilateral intrahippocampal injection of kainate induces a limbic SE that, after a latent period of 1–2 weeks, is followed by highly frequent spontaneous nonconvulsive (electrographic seizures) and less frequent secondarily generalized convulsive seizures in male mice [16]. A similar epilepsy syndrome is observed in female mice [17]; however, to our knowledge, it is not known whether male and female mice differ in duration and characteristics of the latent period. In preliminary experiments, we did not observe any seizure-free ("silent") latent period in female NMRI or C57BL/6 mice following intrahippocampal kainate [18]. Furthermore, the latent period initially reported by Riban et al. [16] in male Swiss mice could not be reproduced in another study in male C57BL/6 mice [19]. Therefore, another aspect that was examined in the present study is mouse strain differences in epileptogenesis. Finally, we studied whether the type of anesthesia (chloral hydrate vs. isoflurane) during intrahippocampal kainate injection affects its consequences.

2. Materials and methods

2.1. Animals

Outbred male and female NMRI (Naval Medical Research Institute) mice, which originated from a colony of Swiss mice and are used as a general-purpose stock in many fields of research including pharmacology [20], female inbred C57BL/6 mice, and female FVB/N mice were obtained from Charles River (Sulzfeld, Germany) at an age of 4–7 weeks (body weight: 20–22 g). Except otherwise indicated, mice were adapted to the laboratory conditions for 1–2 weeks before being used in experiments so that all mice were mid-adolescent at the time of kainate injection. Animals were housed under controlled conditions (ambient temperature: 22–24 °C, humidity: 30–50%, lights on from 6:00 am to 6:00 pm). Food (Altromin 1324 standard diet; Altromin, Lage, Germany) and water were freely available. Female animals were housed without males in order to keep them acyclic or asynchronous with respect to their estrous cycle [cf., 21,22].

Experiments were performed according to the EU Council Directive 210/63/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 (reference numbers for this project: 09/1769 and 14/1659). All efforts were made to minimize both the suffering and the number of animals.

2.2. Intrahippocampal kainate model in mice

In this model, SE is induced by unilateral injection of kainate into the CA1 sector of the dorsal hippocampus [23,24]. For this purpose, mice were anesthetized with chloral hydrate (375–500 mg/kg, i.p.) or isoflurane (3.5% for induction, 1–2% for maintenance of anesthesia; see Results for effects of anesthesia), and kainate (0.21 μg in 50 nl saline, i.e., 1 nM), which was obtained from Sigma-Aldrich (Steinheim, Germany), was stereotaxically injected into the right CA1 area of the dorsal hippocampus as described previously [25]. In some experiments in female NMRI mice, the dose of kainate was reduced to 0.75, 0.5, or 0.25 nM (see Results). Kainate was slowly injected over 60 s with a 0.5-μl microsyringe. In preliminary experiments in groups of 6 mice, stereotaxic coordinates (according to Paxinos and Franklin [26]) were determined for each mouse strain and sex by histological verification of injection site in CA1 (see Table 1). These coordinates were then used for the experiments described in this study. After injection of kainate, the needle of the syringe was maintained in situ for an additional 2 min to limit reflux along the injection track. For EEG recordings, the animals were immediately implanted with bipolar electrodes aimed at the site of kainate injection in the ipsilateral CA1, using the same coordinates as for kainate injection [see 25]. In some experiments, mice were also implanted with cortical EEG electrodes as described previously [27]. Furthermore, basal EEG activity was recorded via hippocampal electrodes in some mice without kainate. During all surgical procedures and for about 1 h thereafter, mice were kept on a warming pad to avoid hypothermia. In mice with isoflurane anesthesia, the isoflurane administration was terminated about 30–60 min after kainate injection, i.e., when all surgical procedures were completed. This led to more rapid recovery of the mice compared to i.p. administration of the long-acting anesthetic chloral hydrate (see Results).

2.3. Video-EEG recording

After surgery, continuous (24/7) video-EEG monitoring started to verify the limbic, predominantly nonconvulsive SE induced by kainate and determine the development of SRS following SE. For this purpose, all mice were continuously video-EEG monitored over 14 days. Four to six weeks after SE, a second period of 3–7 days of continuous video-EEG monitoring was performed to determine the characteristics of SRS in the chronic phase of epilepsy in this model.

For EEG recording, mice were connected via a flexible cable to a system consisting of 8 one-channel bioamplifiers (ADInstruments Ltd., Sydney, Australia) and an analog–digital converter (PowerLab 8/30 ML870, ADInstruments). The data were recorded and analyzed with LabChart 6 for Windows software (ADInstruments) (sampling rate: 200 Hz, time constant: 0.1 s, low-pass filter > 60 Hz, and a 50-Hz notch filter). The EEG recording was directly linked to simultaneous digital video recording using one high-resolution infrared camera for up to eight mice (NYCTO Vision, CaS Business Services, Wunstorf, Germany). For video-EEG monitoring, mice were housed singly in clear plexiglass cages (one per cage) with dark-colored or white-colored bedding, depending on the mouse strain. For monitoring during the dark phase, infrared LEDs were mounted above the cages.

All EEGs were visually examined for abnormal electrographic activity. To quantify the development of epileptogenesis, the occurrence and type of epileptiform activities, as well as their evolution and progressive maturation into high-voltage sharp waves (HVSWs) and hippocampal paroxysmal discharges (HPDs), as described before [16,28], were characterized during the two weeks following KA injection. According to previous studies in epileptic mice [16,28], HVSWs are characterized by low-frequency (3–8 Hz), high-amplitude (1–4.5 mV) sharp waves with a duration of 4–20 s while HPDs are characterized by high-frequency (10–20 Hz), lower-amplitude (0.5–1.1 mV)

Table 1
Stereotaxic coordinates for kainate injection and EEG electrode implantation into the right CA1 of the dorsal hippocampus of mice.

Mouse strain	Sex	Stereotaxic coordinates (in mm from bregma)		
		AP	L	V
NMRI	Male	−2.1	−1.6	−2.3
NMRI	Female	−1.8	−1.6	−1.5
C57BL/6	Female	−1.7	−1.6	−1.9
FVB/N	Female	−1.8	−1.6	−2.0