Epilepsy Research (2013) **105**, 280—291

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

Spontaneous seizures in a rat model of multiple prenatal freeze lesioning

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Received 23 September 2012; received in revised form 8 February 2013; accepted 15 March 2013
Available online 29 April 2013

KEYWORDS
Focal cortical dysplasia;
Freeze lesion rat model;
Temporal lobe epilepsy.

Summary Focal cortical dysplasia (FCD) is an important cause of intractable epilepsy. Previous rat studies have utilized freeze lesioning of neonatal animals to model FCD; however, such models are unable to demonstrate spontaneous seizures without seizure-provoking events. Therefore, we created an animal model with multiple FCD, produced during embryonic development, and observed whether spontaneous seizures occurred. Furthermore, we examined the relationship between FCD and epileptogenesis using immunohistochemistry.

At 18 days postconception, a frozen metal probe was placed bilaterally on the scalps of Sprague-Dawley rat embryos through the uterus wall to produce multiple FCD. Electroencephalogram (EEG) and video recording were performed from postnatal day (P) 35 to P77. Brain tissues were examined immunohistochemically at P28 and P78 using semiquantitative densitometry. Eleven of 16 rats (68.8%) showed spontaneous seizures arising in the hippocampus from P47. Movement cessation followed by sniffing and mastication, culminating in wet-dog shaking, was seen during the hippocampal EEG discharges. FCD was observed in the bilateral frontoparietal lobes. The expression levels of *N*-methyl-D-aspartate receptor (NMDAR) subunits 1, 2A, 2B, the glutamate/aspartate transporter and the glial glutamate transporter 1 (GLT1) at FCD sites were increased at P28 and P78. There were no major histological abnormalities in the hippocampi compared with those in the cortex. However, the expression levels of NMDAR 2A and 2B were increased at P28. Levels of NMDAR1, 2A and 2B, the glutamate/aspartate transporter and GLT1 were also increased at P78.

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0920-1211/$ — see front matter
http://dx.doi.org/10.1016/j.eplepsyres.2013.03.003

We created an animal model showing spontaneous seizures without a provoking event except for the existence of cortical dysplasia, and without a genetic or general systematic cause like MAM injection or irradiation. The seizures resembled human temporal lobe epilepsy both clinically and on EEG. Alterations in the levels of glutamatergic and GABAergic receptors were investigated during growth. This model should enable better clarification of the mechanisms underlying the development of human epilepsy.

Introduction

Focal cortical malformation is occasionally detected in patients receiving surgical treatment for intractable epilepsy (Fauser et al., 2004). Based on histology, focal cortical malformations are classified into focal cortical dysplasia (FCD) and heterotopias (Palmini et al., 2004). FCD is characterized by abnormal cortical organization with or without cellular abnormalities, and is considered closely related to intractable epilepsy (Blümcke et al., 2011).

In vitro studies using brain slices prepared from the human dysplastic neocortex demonstrated an intrinsic hyperexcitability (Mattia et al., 1995; Avoli et al., 1999; Cepeda et al., 2003). In addition, electroencephalography (EEG) and magnetoencephalography revealed intrinsic epileptogenicity in the magnetic resonance imaging (MRI)-proven dysplastic cortex (Morioka et al., 1999; Bast et al., 2004; Otsubo et al., 2005). Furthermore, intralesional electrocorticogram showed interictal and ictal epileptic activities (Francione et al., 2003). Nevertheless, FCD can also be detected on MRI without apparent neurological abnormalities (Kasper et al., 1999). As such, the mechanism responsible for epileptogenesis of FCD remains undetermined.

In the human, cortical dysgenesis is caused by insults such as hypoxia (Richman et al., 1974) and ischaemia (Ferrer, 1984) during corticogenesis in the prenatal or perinatal period. In experimental animal models, malformation of the cortex during development is induced by irradiation or methylazoxymethanol (MAM) injection of embryos (Kondo et al., 2001; Kellinghaus et al., 2004; Colcitti et al., 1998; Germano and Sperber, 1998; Harrington et al., 2008). Genetic knockout animal models of tuberous sclerosis also show cortical malformation (Meikle et al., 2007; Zeng et al., 2008). These animal models show spontaneous seizures; however, the insults are general or genetic. In contrast with these models, a relatively focal, not general or genetic, insult is given in the freeze-lesion model. In experimental freeze-lesion rat models, cortical malformation created by neonatal freeze lesioning shows extra-sulci and microgyri, while cortical hyperexcitability was revealed by only ex vivo slice studies (Jacobs et al., 1996; Luhmann and Raabe, 1996). However, neither clinical nor electroencephalographic seizures have been observed in the rat neonatal freeze-lesioning model (Scantlebury et al., 2005; Kellinghaus et al., 2007).

In rodents, migration of cortical neurons is largely completed by birth (Bayer and Altman, 1995). Therefore, we previously created an experimental FCD rat model using prenatal freeze lesioning, which developed FCD in one hemisphere. The FCD in this model displayed severe disorganization of the cortical layers with randomly oriented dendrites and axons. Furthermore, significantly prolonged after-discharges were observed in the cortex and hippocampus compared with those in sham-operated control rats, as well as early development of hippocampal kindling. *N*-methyl-D-aspartate receptor (NMDAR) 2B immunoreactivity was significantly enhanced in the cortex following freeze lesioning (Takase et al., 2008). However, this model lacked spontaneous epileptic seizures.

In the present study, we created a rat model with multiple FCD in the bilateral hemispheres by freeze lesioning during the late embryonic stage. The aim of our study was to clarify whether bilateral multiple FCD lesions induce spontaneous EEG and clinical seizures without cortical electrical kindling stimulation.

Materials and methods

Ethical statement

Experimental procedures were designed to minimize the number of animals used and to minimize animal suffering. All animal experiments were carried out according to the guidelines for proper conduct of animal experiments published by the Science Council of Japan, and ethical approval for this study was granted by the Animal Care and Use Committee of Kyushu University.

Rat model of focal cortical malformation by freeze lesioning of embryos

Pregnant Sprague-Dawley (SD) rats (Charles River Laboratories, Sherbrooke, Quebec, Canada) were used. In all groups, pregnant SD rats were anaesthetized with pentobarbiturate (50 mg/kg, intraperitoneal injection) at 18 days postconception (embryonic day 18: E18). Lidocaine was injected into the lower abdominal skin, abdominal longitudinal muscles and peritoneum. Bilateral uteri were partially extracted from the abdominal cavity through a longitudinal incision. A metal probe with a hemispheric tip that had a diameter of 2.3 mm, cooled by liquid nitrogen, was placed onto the scalp of a rat embryo from outside of the uterus wall for 4 s After the operation, uteri were returned into the abdominal cavity and the cavity was filled with 0.9% saline. The peritoneum, muscles and skin were sutured. Mother rats were returned to their cages and warmed until they recovered from the anaesthesia, and were then handled under normal conditions until delivery. Rat pups were born at E22 and reared by the mother until postnatal day (P) 28.

Rat pups were grouped as follows: Group A, bilateral multiple lesions (two longitudinal lesions on each hemisphere, $n = 21$); Group B, bilateral single lesions (one lesion on each