Pathophysiological Analysis of the Epileptogenic Zone in Familial Spontaneous Epileptic Cats: Electroencephalographic, Imaging, and Pathological Studies

Summary of Doctoral Thesis

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Epilepsy develops spontaneously in various animal species and is the most common cerebral disease in human and veterinary medicine. In veterinary clinical medicine, the development of idiopathic epilepsy is very rare in cats, and feline genetic epilepsy has not been reported. In 2009, Kuwabara and Hasegawa identified familial spontaneous epileptic cats (FSEC) in which the development of epilepsy was speculated to be inherited in an autosomal recessive manner. FSEC exhibit 2 types of seizure: spontaneous limbic seizures with secondarily generalization that strikingly resemble kindling and kainic acid-induced epilepsy models in cats; and generalized tonic-clonic seizures induced by vestibular stimulation that closely resemble those observed in EL mice. Since the kindling and kainic acid-induced models in cats and EL mice are animal models of human mesial temporal lobe epilepsy (MTLE), FSEC are also considered to be an animal model of human MTLE.

Lűders et al. proposed the presence of the following 6 types of abnormal regions including the epileptogenic zone: 1) structural abnormal zone or epileptogenic lesion, which is a macroscopic lesion causing epileptic seizures by hyperexcitation of the lesion or its adjacent area that is demonstrated by morphological imaging such as high magnetic field magnetic resonance imaging (MRI); 2) functional deficit zone, which is a region with abnormal function during an interictal period that is demonstrated by functional imaging such as functional MRI (diffusion-weighted imaging and perfusion-weighted imaging); 3) electroencephalographic (EEG) abnormal zone or initiative zone, which is a region in which paroxysmal discharges are observed in an interictal period that is mainly assessed by EEG; 4) ictal-onset zone, which is a seizure-originating region that is mainly assessed by EEG; 5) symptomatogenic zone, which is a region inducing the early symptoms and signs of seizures that is determined symptomatologically in the early stage of seizures; and 6) epileptogenic zone, which is required to induce epileptic seizures and seizures can be suppressed by its resection. These conceptual zones are not necessarily located in an identical position in every case and their interrelation also differs from case to case. In the present study, we identified the epileptogenic zone and performed pathophysiological analysis using electroencephalogical, diagnostic imaging, and histopathological techniques in FSEC based on the concept of these abnormal epileptic zones.

1. Chronic depth EEG analysis and pathological analysis in FSECs (Chapter II)

The most fundamental method for the pathophysiological analysis of epilepsy is the localization of an epileptic focus by EEG recordings. This chapter describes the results of the analysis of ictal EEGs by stereotactic implantation of depth electrodes and subsequent long-term video EEG monitoring in 5 FSECs. These FSECs were also analyzed by conventional pathological methods. In this study, subclinical and clinical spontaneous seizure activity was observed 54 times in total.

Clinically observed focal and secondarily generalized seizures were the typical limbic seizures detected and were synchronized with amygdaloid and/or hippocampal epileptiform activity; therefore, their symptomatic zone and ictal onset zone were suggested to exist in the amygdala and hippocampus.

However, the localization of the focus and laterality in subclinical and clinical focal ictal activity differed between individuals. A slight decrease in the number of neuronal cells was observed in the pyramidal cell layer of the hippocampus by histopathological analysis. It is considered, however, that further detailed assessment in intact cats is necessary because the influence of electrode insertion cannot be excluded completely.

2. 3D hippocampal volumetry of FSECs using high magnetic field MRI: time course changes in hippocampal volume (Chapter III)

The measurement of hippocampal volume (HV) by MRI is useful for the detection of hippocampal atrophy and laterality in the epileptogenic lesion of human MTLE patients and has been used commonly for the diagnosis and noninvasive preoperative assessment of hippocampal sclerosis (HS). The purpose of the studies described in this chapter was to detect structural abnormal zones in FSEC by 3D MR hippocampal volumetry. In addition,

long-term changes in HV were assessed in 10 FSECs. HV was measured in 18 FSECs and compared with non-age matched control cats, and it was also measured in 14 FSECs and compared with age-matched control cats. In both studies, significant hippocampal asymmetry was found in FSECs, but no significant difference was found in HV between the FSECs and control groups. The age-matched experiment, however, demonstrated that the smaller side of HV in FSECs was significantly reduced compared to mean unilateral HV in controls. In addition, biennial long-term measurements of HV (3 times in total) in 10 FSECs revealed a tendency for a long-term reduction of HV and increase in hippocampal asymmetry, although these differences were not significant. This study demonstrated the presence of a structural abnormal zone in the hippocampus of FSECs; therefore, it is considered that the identification of hippocampal atrophy is useful for the non-invasive diagnosis of an epileptic focus.

3. Functional imaging analysis by diffusion and perfusion imaging using high magnetic field MRI: changes during interictal periods and immediately after seizures (Chapter IV)

Functional imaging analysis can be used as a method for noninvasive focal diagnosis in epilepsy to assess pathology and cerebral function from an aspect that is different from, but complementary to, electrophysiological and morphological assessments. This chapter describes the results of the study of imaging parameters during interictal periods in FSECs as measured by diffusion and perfusion MRI. In some FSECs exhibiting induced seizures, diffusion and perfusion MRI was also performed immediately after seizures, and the changes in imaging parameters between interictal and postictal periods were compared. In FSECs, a significant elevation of diffusibility (ADC value) and reduction of fractional anisotropy (FA value) were found in the hippocampus and amygdala during interictal periods, suggesting that there were microscopic changes in tissue structure such as the expansion of extracellular space in these regions. On the other hand, hypoperfusion was

observed in the hippocampus, amygdala, and cerebral cortex, suggesting the presence of a functional deficit zone in these regions. In postictal periods, low diffusion and hyperperfusion areas were observed in and around the hippocampus and amygdala, suggesting the influence of ictal activity and its propagation. These observations were consistent with the origin of seizures in the hippocampus and amygdala on intracranial EEG recordings (Chapter III). In FSECs, the results of diffusion and perfusion MRI indicated a functional deficit zone during interictal periods and possibly reflected epileptogenic foci during postictal periods; therefore, this method is considered to be useful as an approach for the noninvasive diagnosis of epileptogenic foci.

4. Histopathological analysis of the hippocampus and amygdala in FSECs (Chapter V)

This chapter describes the results of the assessment of neuronal cell numbers and gliosis, which are findings for HS in human MTLE patients, in intact homozygous FSECs developing seizures and a small number of heterozygous FSECs that were parents of FSECs. “Intact FSECs” means FSECs not subjected to invasive surgery such as electrode insertion. The results of the examination of granular cell dispersion (GCD) and mossy fiber sprouting (MFS) in the hippocampus are also described. Extensive neuronal cell loss was observed in the pyramidal layer of the hippocampus and amygdala of homozygous and heterozygous FSECs, but this decrease was not associated with gliosis. This finding was not consistent with the typical findings for HS in human MTLE patients. In homozygous FSECs, MFS was not found, but gliosis without neuronal cell loss in the CA4 region of the hippocampus and GCD-like findings characteristic for HS were observed. The hippocampal abnormality in FSEC represented by neuronal cell loss without gliosis was suggested to be the result of a malformation that is different from typical HS, such as human familial mesial temporal lobe epilepsy (FMTLE).

For the identification of the epileptogenic zone and pathophysiological analysis of FSECs, electrophysiological, diagnostic imaging, and histopathological studies were conducted. Video EEG recordings with depth electrodes demonstrated epileptiform EEG activity (ictal onset zone/symptomatogenic zone) in the hippocampus and amygdala, and the measurement of HV and functional imaging revealed a macroscopic structural abnormality in the hippocampus (structurally abnormal zone) and microscopic structural changes with lowered function (functional deficit zone), respectively. Pathological investigation also demonstrated a decrease in the number of neuronal cells in the hippocampus and amygdala. These results suggested the presence of an epileptogenic zone in the hippocampus and amygdala in FSEC and proved that epilepsy in FSEC was homologous with human MTLE. To prove the epileptogenic zone in FSEC, surgical resection and confirmation of seizure free should be performed in the future study.

The frequency of seizures, their severity, and the location and laterality of the epileptogenic zone differed between individual FSECs, and these heterogeneous phenotypes are also observed in human FMTLE patients. On the basis of these clinical and pathological similarities between FSEC and human FMTLE patients, we have no choice but to consider the possibility that the hippocampal atrophy and decreased number of neuronal cells found in FSEC are also congenital/genetic malformations. Furthermore, similar pathological changes were observed in heterozygous FSECs that had not developed epilepsy. Therefore, it is unlikely that only these pathological changes in the hippocampus are involved in the onset of seizures, and a variety of etiologies are considered to be involved in epileptogenicity in FSEC. Thus, the mode of inheritance in FSEC may not be simple autosomal recessive inheritance as was first supposed upon the discovery of this animal model, and genetic analysis to identify the responsible genes will be required in the future. FSEC are the sole feline model of genetic epilepsy and also represent a unique model in veterinary medicine. FSEC can be a good animal model of MTLE as well as a kindling model, a kainic acid model, and EL mice, and especially it may be a specific model of human FMTLE. Since human FMTLE also has a variety of phenotypes, the

responsible genes have not been identified. Therefore, the identification of the responsible genes in FSEC can have an important role in the elucidation of the pathophysiology of human FMTLE and feline epilepsy. It is considered that the elucidation of the pathogenic mechanism for epilepsy in FSEC will play a in the analysis of the pathophysiology of epilepsy that is common between veterinary and human medicine.