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Functional imaging and pathology in brain of interictal cats kindled by pentylenetetrazol

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Abstract

Objective: To investigate the electroencephalogram (EEG) characteristics, cerebral blood flow, glucose metabolism, and pathological characteristics in cats kindled by pentylenetetrazol (PTZ). *Methods:* Ten adult male cats received intramuscular injections of PTZ at a sub-convulsive dose of 25 mg/kg once daily. Cats were considered to be kindled when a class V or above seizure was observed. Subsequently, scalp EEG, 99mTc-ECD-single-photon emission computed tomography (SPECT), 18FDG-positron emission tomography (PET), and Cresyl violet staining were employed to evaluate brain activity, cerebral blood flow, glucose metabolism, and pathological characteristics representing epileptic foci during interictal stages. *Results:* The EEG data revealed bilateral dissymmetry paroxysmal activity in the form of δ and θ waves, as well as paroxysmal spikes and sharp waves instead of symmetric high-amplitude rhythm of 8-12 Hz in cats kindled by PTZ compared to controls. In addition, low blood perfusion and low glucose metabolism unilaterally in the temporal lobe were observed in PTZ-kindled cats, in contrast to the bilateral symmetrical blood perfusion and high glucose metabolism observed in control cats. Pathological examinations showed a thickening of white matter in the ipsilateral temporal region and a thinning of gray matter in PTZ-kindled cats. Microscopically, we observed a structure disturbance of hippocampal CA3 area in PTZ-kindled cats.
Conclusion: Epileptic foci of PTZ-kindled cats were localized to the unilateral temporal lobe, in a manner highly similar to the pathophysiology of human temporal lobe epilepsy.

INTRODUCTION

Animal seizure models provide useful evidence related to human seizure that can be obtained from clinical practice; such models have therefore played a fundamental role in our understanding of the basic mechanisms that underlie epileptogenesis. An improved overall understanding of seizure characteristics in a diversity of animal models will therefore facilitate the study of human seizures.[1] The pentylenetetrazol (PTZ) kindling model is an ideal tool for the modeling of focal to generalized seizure attacks. However, the location of epileptic foci following PTZ administration, the impact upon measures of functional brain imaging, and the resultant pathological characteristics of PTZ-induced kindling in this model remain poorly understood.

METHODS

Animals and treatment

All experiments involving animals were approved by the Animal Ethics Committee at the Chinese Academy of Sciences. Twelve adult male cats (weighing 3.0-3.5 kg) were purchased from the Tairi Experimental Animal Center of Fengxian District (Shanghai, China). Each cat was maintained in a cage with dimensions of 0.8 m × 0.6 m × 0.6 m. Ten cats in the experimental group were administered intramuscular injections of PTZ once daily at a sub-convulsive dose of 25 mg/kg.[2] Two cats in the control group were treated identically to the experimental group, except that they received equal volume of normal saline instead of PTZ. All experiments were performed at the same time of day.

Behavior observations

A three-hour observation period commenced immediately following the PTZ or saline injections each day, which included measures of facial motion, head motion, standing, walking, eating, and vocalizing. The attack time and duration of different classes of seizure were also recorded.

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The pattern of seizure development was classified according to the criteria established by Wada.[3] In brief, these included six stages: Stage 1, ipsilateral facial twitching; Stage 2, bilateral facial twitching; Stage 3, head nodding; Stage 4, tonic extension of the contralateral forepaw; Stage 5, clonic jumping while standing; and Stage 6, falling down with generalized tonic-clonic convulsion. Cats were considered to be kindled when a seizure of stage 5 or above seizures was observed. Once kindling was established, the PTZ administration ceased.

MR brain imaging of PTZ kindled cats

A Siemens 1.5T MR imaging system was employed for the brain imaging of cats before and after kindling. Kindled animals were imaged after they had experienced five seizure attacks at Stages 5 or 6.

Electroencephalogram (EEG) recording of PTZ-kindled cats

Baseline EEG recordings from all 12 cats prior to PTZ or saline administration were obtained using a digital polygraph (Medlec: Profile).Upon experiencing five Stage 5 or 6 seizures following kindling, EEG profiles of PTZ-treated cats were recorded during the interictal, or between-attack, period two hours following the most recent attack. The acquisition parameters were as follows: time constant, 20 min; high filter, 15Hz; low filter, 0.5Hz; sensitivity, 50-100. All cats were anesthetized with ketamine (15 mg/kg) prior to EEG recording and then a monopolar stainless-steel EEG electrode was placed bilaterally over the frontal, temporal, parietal, and occipital regions. A neutral electrode was placed the central of the eyebrow.

Measurements of cerebral blood flow perfusion using single-photon emission computed tomography (SPECT) imaging

We measured baseline cerebral blood flow perfusion in cats prior to treatment with PTZ or saline using a Siemens e.cam Dual Detector SPECT with the imaging agent99mTc-ethylcysteinate dimer (99mTc-ECD) and a probe equipped with a high fan beam collimator apparatus. Following PTZ or saline administration and the establishment of kindling, SPECT images were obtained from 10 kindled cats during the interictal period (2 hours following the 5th attack, as with the EEG recording) and 2 control cats. The imaging agent 99mTc-ECD was purchased from Xinke Pharmaceutical Co., Ltd (Shanghai, China). All cats were anesthetized with ketamine (15 mg/kg) prior to imaging. To block the signal from the choroid plexus, each cat was administered 200 mg potassium per chlorate for 15 min prior to the forelimb intravenous injection of 99mTc-ECD 1110MBq (30mCi). Imaging commenced 15 minutes following the 99mTc-ECD injection. The parameters of the acquired images were as follows: 128 × 128 matrices, each frame covered 360°, stepping 6°, 60 frames acquired, 2.29 zoom, 140KeV energy peak, 20% energy window, 80K/frame and brain counting 2.0k/s. A different cut frequency (0.48) and order (20) of Butterworth were used for image reconstruction. Images were corrected using Chang's attenuation correction method (attenuation coefficient=0.15). The cross section was parallel to line OM. Images were double-blind analysis by two nuclear medicine physicians independently.

Positron emission tomography (PET) imaging of cerebral metabolism

18F-fluorodeoxyglucose (FDG) with a purity of >95% was synthesized in our hospital using a RDS111 cyclotron and a hot cell (CTI, Inc., Springfield, Illinois, USA). All PET studies were carried out using a Siemens ECAT EXACT HR+ PET scanner (CTI, Knoxville, USA) with an axial field of view of 16.2 cm and a central axial and transversal direction of 4.2 mm and 4.6 mm, respectively. Cats were placed in the PET scanner five minutes after a forelimb intravenous injection of 18F-FDG (0.5-0.6mCi). Adjustments were made to bring the brain into the center of the field of view. The image acquisition parameters were as follows: emission scanning with 30,000,000 counting presupposition followed by transmission scanning with five minute time presupposition. Images were corrected with the T and E attenuation correction method and then reconstructed to acquire cross-sectional, coronal, and sagittal plane images. The parameters of the filtered back projection (FBP) reconstruction included 128 × 128 matrices, 5.0 zoom, a RAMP window function of transversal direction with a cut frequency of 0.5, and a function of axial direction with a cut frequency of 0.5.

Cerebral histological observations

Following the EEG, SPECT, and PET experiments, all cats were anesthetized with 4 ml of ketamine, transcardially perfused with with 4% paraformaldehyde, and decapitated to obtain brain tissues. The white matter ratio (widest

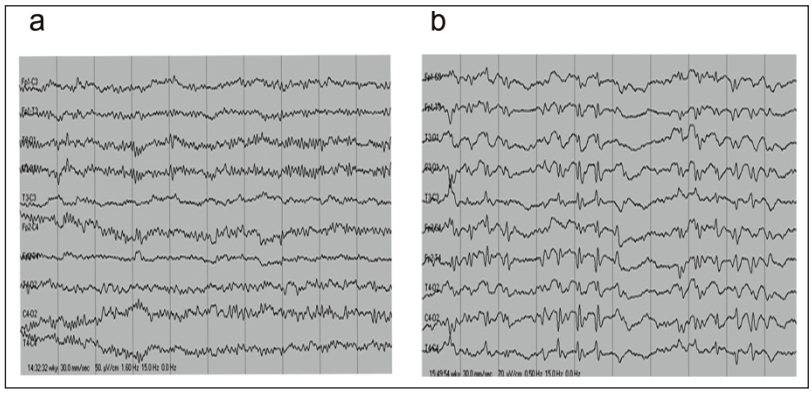

Figure 1. EEG of control and PTZ-kindled cats during interictal stages. (a). Symmetrical high-amplitude rhythm of 8-12 Hz with no paroxysmal spikes or sharp waves were observed in control cats. (b). Bilaterally dissymmetrical paroxysmal spikes and sharp waves were present in PTZ-kindled cats.

white matter tract / length from brain edge to the midline through the widest white matter tract) was used to evaluate differences between the two cerebral hemispheres in PTZ-kindled cats and control cats. Tissue sections were submitted to routine hematoxylin-eosin (HE) and Cresyl violet staining, and examined using a light microscope with a magnification of 20X.The quantitative analysis of cell number in hippocampal area CA3 was performed by calculating the average number of cells present in five representative fields from each section.

RESULTS

Behavioral observations

The latency between the first PTZ injection and the onset of kindling ranged from two to three weeks, with a median time of 20±2 days. The observed behavioral abnormalities increased gradually the period of kindling induction. All kindled cats show clonic jumping while standing (Stage 5) or falling down with generalized tonic-clonic convulsion (Stage 6). The two cats in the control group received intramuscular injections with normal saline, and no significant behavioral changes were observed in these animals.

EEG observations

Prior to injection with PZT or normal saline, each of the 12 cats was anesthetized with ketamine for the EEG recording. This baseline EEG data displayed symmetrical high-amplitude rhythms at 8-12 Hz, and no paroxysmal spikes and sharp waves were observed (Figure 1 a). However, following PTZ-induced kindling, EEG recordings during the interictal period revealed bilaterally dissymmetric paroxysmal spikes and sharp waves, in addition to a generalized slowing of the background rhythm (Figure 1 b).

MR brain imaging of PTZ-kindled cats

MR images of cat brains before and after PTZ-induced kindling were acquired using the Siemens 1.5T MR imaging system. No structural changes in the animals' brain were observed following PTZ-induced kindling (Figure 2).

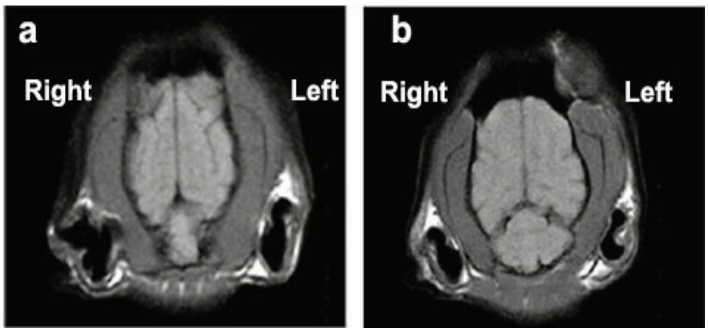

Figure 2. MR brain imaging of cats before and after kindling induced by PTZ treatment. There were no structural abnormalities observed in cats either before PTZ treatment (a) or following PTZ-induced kindling (b).

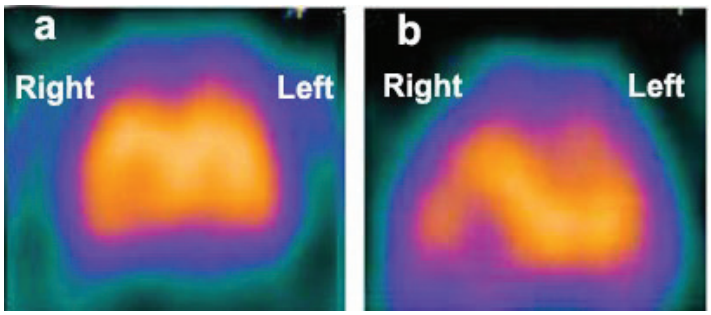

Figure 3. SPECT imaging of cats before treatment with PTZ and following PTZ-induced kindling. (a). The bilateral cerebral blood flow perfusion was symmetrical in cats prior to treatment with PTZ. (b). The cerebral blood flow perfusion was significantly lower in the right temporal lobe compared with that in the left temporal lobe using SPECT in a representative PTZ-kindled cat.

Cerebral SPECT and PET characteristics of PTZ-kindled cats during interictal stages

The cerebral SPECT results revealed unilateral low blood perfusion in the (right, 6 cats; left, 4 cats) temporal lobe in PTZ-kindled cats during the interictal period (Figure 3). Similarly, low glucose metabolism was evident in the ipsilateral temporal lobe using PET (Figure 4). Symmetric blood perfusion and glucose metabolism was observed in the control cats.

Pathological characteristics of PTZ-kindled cats

In the PTZ-kindled cats, it was evident to the naked eye that the gray matter was thinner and the white matter was thicker. Our white matter ratio analysis was performed in the unilateral temporal lobe, defined as the side which showed sharp-slow waves by scalp EEG, low blood perfusion by SPECT, and low glucose metabolism by PET in the PTZ-kindled cats. No changes in white matter ratio were observed in the contralateral temporal lobe of PTZ-kindled cats, or in either side of the control brains (Figure 5). Microscopically, Cresyl violet staining revealed no significant abnormalities in the gross morphology of the hippocampal dentate gyrus on the side of low blood perfusion compared to the contralateral side in the PTZ-kindled cats (Figure 6 a, c). However, structural disturbances and a loss of layer structure were evident in the ipsilateral hippocampal area CA3 compared to the contralateral side in the PTZ-kindled cats (Figure 6 b, d). The number of neurons in area CA3 did not differ significantly between the two hemispheres in the PTZ-kindled animals (Figure 6 e)

DISCUSSION

In the current study, interictal EEG measurements displayed sharp-slow waves in the unilateral temporal lobe of PTZ-kindled cats in addition to SPECT and PET measurements indicating low blood perfusion and glucose metabolism, respectively, in the same side. Together, these

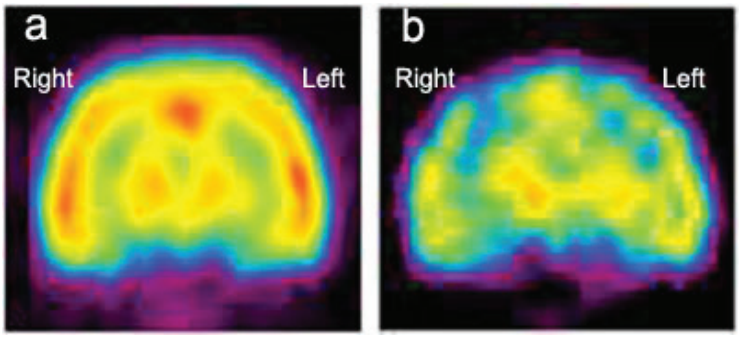

Figure 4. PET imaging of cats before treatment and following PTZ induced kindling. (a). Bilateral cerebral glucose metabolism was symmetrical in cats prior to treatment with PTZ. (b). Glucose metabolism in the right temporal lobe was significantly lower than that in the left in PET images from a PTZ-kindled cat.

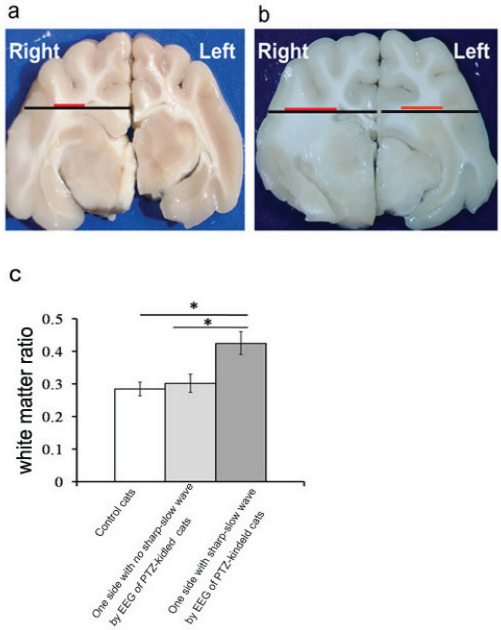

Figure 5. Structure of the temporal lobe in control and PTZ-kindled cats. (a). Bilateral structure of the temporal lobes of the control cats are basically symmetrical. (b). In a represent PTZ-kindled cat, white matter in the right temporal lobe was thickened, and the gray matter thinned, compared to the left temporal lobe. (c). The ratio of the widest white matter and the length from brain edge to the midline through the widest white matter in hippocampal coronal sections of control cats and both sides of PTZ-kindled cats. *, $p<0.05$.

findings suggested that epileptic foci were located unilaterally to the temporal lobe of PTZ-kindled cats.

The PTZ-induced kindling model is a classic experimental representation of temporal lobe epilepsy. It has been widely used in the study of epileptogenic mechanisms and anti-epileptic drugs discovery.[4-8] However, to date, the exact locations of epileptic foci in animals subjected to PTZ-induced kindling are still poor understood. By using SPECT and PET imaging technologies, combined with EEG and pathological evaluation, the current study found the location of seizure foci in PTZ-kindled cats to reside unilaterally in either side of the temporal lobe.

The mechanism of action of PTZ involves the function of GABA receptors at the synapse, where PTZ acts to decrease the inhibitory capacity of GABA signaling.[9] PTZ produces rapid onset of seizures when given intravenously at relatively high concentrations.[8,10-11] Intraperitoneal administration initiates seizure activity after a period of delay[12], which may be related to the gradual increase of blood levels of the drug. However, the anatomic localization of PTZ activity following administration has not been characterized completely.

In general, epileptic foci consist of several pacemaker neurons, which can generate paroxysmal epileptiform activity. At the level of a single neuron, the cellular correlate of an interictal epileptiform discharge is the paroxysmal depolarization shift (PDS). The PDS is characterized by a prolonged calcium-dependent depolarization that results in multiple sodium-mediated action potentials during the depolarization phase, and it is followed by a prominent after-hyperpolarization, defined as a hyperpolarized membrane potential beyond the baseline resting potential.[13] High-frequency oscillations, termed fast ripples, have been identified in seizure-generating limbic areas in rats made epileptic by intrahippocampal injection of kainic acid.[14,15] Interictal high-frequency oscillations (80-500 Hz) have also been observed in the entorhinal cortex of the epileptic human brain accompanied by EEG recordings that show intermission firing, which is not concomitant seizures attack.[16] The center and surround of epileptic foci have also been recorded to show hyperpolarized potentials during epileptic firing in the cortex. Studies in animal models indicate that this hyperpolarized potential results in the spike-and-slow-wave complex.[17] In these studies,

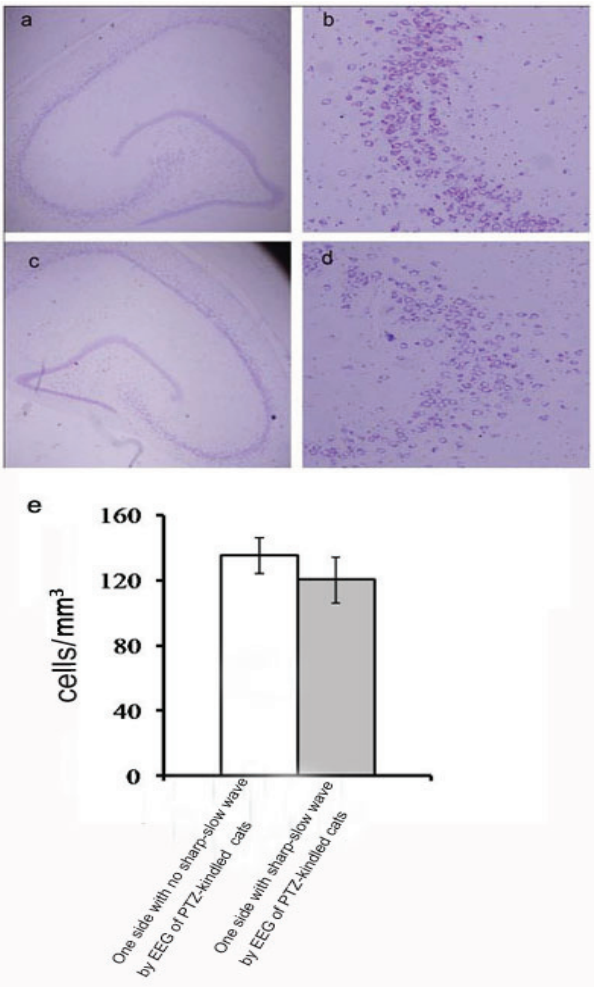

Figure 6. Cresyl violet staining of hippocampus in a PTZ-kindled cat. (a), (c). Gross morphology of the left (a) and right (c) hippocampus of a PTZ-kindled cat (10 X). (b), (d). Pathological changes of hippocampal CA3 area in the left (a) and right (b) side of the PTZ-kindled cat brain (20X). (e). Quantitative analysis of cell number in both sides of hippocampal CA3 area in PTZ-kindled cats.

sharp waves, and low blood perfusion and glucose metabolism were observed in these epileptic foci. Additionally, neuronal hypofunction has been shown in these foci compared with normal brain.

In the current study, the cerebral pathological characteristics of PTZ-induced kindling in cats included abnormal EEG, low blood perfusion, and low glucose metabolism unilaterally in the temporal lobe. However, no structural changes in the animals' brain were observed in MRI following PTZ-induced kindling. It is possible that the field strength of the MRI was too low to discriminate structural abnormalities. In addition, through histological observations, we founded white matter thickening and gray matter thinning in the ipsilateral temporal region by naked eyes, as well as the structure disturbance of hippocampal area CA3 by microscopy. However, no significant difference in the number of neurons or nerve fibers was observed in the PTZ-kindled brain. These results suggest that abnormal neuronal connectivity may play a vital role.

In conclusion, we used a relatively large mammalian adult cat model to demonstrate behavioral changes and abnormalities in measures of EEG, cerebral blood perfusion, glucose metabolism, and histopathology following PTZ-induced kindling, which suggest that epileptic foci of PTZ-kindled cats were localized to the unilateral temporal lobe. The findings of the current study may provide useful information for the study of epileptogenic mechanisms, anti-epileptic drug discovery, and optimal treatment strategies.

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DISCLOSURE

Conflict of interest: None

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