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IN VIVO MAPPING OF TEMPOROSPATIAL CHANGES IN GLUCOSE UTILIZATION IN RAT BRAIN DURING EPILEPTOGENESIS: AN ^{18}F-FLUORODEOXYGLUCOSE–SMALL ANIMAL POSITRON EMISSION TOMOGRAPHY STUDY

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Abstract—Cerebral glucose hypometabolism is common in temporal lobe epilepsy (TLE). The temporospatial evolution of these metabolic changes during epileptogenesis remains to be determined. We measured the regional normalized cerebral metabolic rate for glucose (nCMRglc) with ^{18}F-fluorodeoxyglucose (FDG)–small animal positron emission tomography (microPET) in animals receiving systemic pilocarpine application. The microPET scan was performed on day 2 (early), day 7 (latent) and 42 days (chronic phase) after the initial status epilepticus. We found specific temporospatial changes in glucose utilization in rats during the course of epileptogenesis. In the early phase, the limbic structures underwent the largest decrease in glucose utilization. Most brain structures were still hypometabolic in the latent phase and recovered in the chronic phase. Conversely, the hippocampus and thalamus presented with persistent hypometabolism during epileptogenesis. The cerebellum and pons maintained normal glucose utilization during this process. We also found that severe glucose hypometabolism in the entorhinal cortex during the early phase was correlated with epileptogenesis, indicating the critical role of the entorhinal cortex in the early stages of TLE.

Key words: pilocarpine, positron-emission tomography, glucose utilization, epilepsy, entorhinal cortex.

Cerebral glucose hypometabolism is common in human temporal lobe epilepsy (TLE) and is frequently specific enough to identify seizure onset zone in patients (Boling et al., 2008; Liew et al., 2009; Vinton et al., 2007). Hypometabolism may not only be a chronic accompaniment of the epileptic brain, but also a direct contributor to epileptogenesis (Pan et al., 2008). Several metabolic mapping studies have investigated the relationship between local cerebral glucose metabolism and epileptogenesis in animal models using [^{14}C]2-deoxyglucose autoradiography (Dubé et al., 2001; Fernandes et al., 1999). This method, however, is performed on postmortem tissue and is therefore limited to a single time point of the epileptogenic process (Dedeurwaerdere et al., 2007). The temporal evolution of the metabolic changes in the brain during epileptogenesis remains to be determined, and there is a need for noninvasive *in vivo* imaging techniques to map it.

Small animal positron emission tomography (microPET) is a noninvasive imaging technique that allows *in vivo* monitoring of cerebral metabolic patterns in the rat brain (Gao et al., 2009; Lou et al., 2007). The usefulness and feasibility of *in vivo* [^{18}F]-fluorodeoxyglucose (FDG)–microPET imaging in animal models of epilepsy has been demonstrated in a model of kainic acid (KA)–induced seizures (Kornblum et al., 2000) and in a pilocarpine model of TLE in C57BL/6 mice (Mirrione et al., 2006). Both these studies show markedly increased glucose metabolism during status epilepticus (SE) in several brain areas. Recently, a longitudinal microPET imaging study showed global hypometabolism persisting 6 weeks after KA-induced SE, where this was not correlated with the presence of spontaneous recurrent seizures (SRS) (Jupp et al., 2007). The glucose metabolism of the whole brain, however, was analyzed instead of focusing on specific brain regions. Goffin et al. (2009) reported that pilocarpine-induced SE caused a similar global hypometabolism in the early epileptogenic stage in the rat brain. They found metabolic differences in some brain areas between animals which did and did not develop SRS; however, this finding was variable due to the very small number of animals sampled. Hence, the temporospatial changes in glucose metabolism in the rat brain during epileptogenesis have yet to be elucidated.

Epileptogenesis is very difficult to study in humans, as the chronic rather than the early epileptogenic stage is found in most patients. Systemic administration of pilocarpine causes limbic seizures and SE in rats, followed by the latent phase with no apparent seizure activity and then the chronic phase with SRS (Curia et al., 2008). This animal model reproduces many of the clinical and histopathological features of human TLE (Curia et al., 2008),

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Abbreviations: CMRglc, regional glucose metabolic rate; EEG, electroencephalographic; FDG, [^{18}F]-fluorodeoxyglucose; KA, kainic acid; microPET, small animal positron emission tomography; nCMRglc, normalized regional glucose metabolic rate; PN, postnatal day; ROI, region of interest; SE, status epilepticus; SRS, spontaneous recurrent seizures; TLE, temporal lobe epilepsy.

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and allows for the study of epileptogenic processes during the latent phase.

The present study aimed to investigate the temporo-spatial changes in glucose metabolism in the rat brain at three different time points after pilocarpine administration using FDG–microPET. The time points were the early phase (2 days after SE onset), the latent phase (7 days after SE onset) and the chronic phase (42 days after SE onset). The data obtained were correlated with the duration of the latent phase and frequency of SRS in the early stages of the chronic phase. This allowed for the analysis of the relationship between the dynamic cerebral metabolic changes and epileptogenesis.

EXPERIMENTAL PROCEDURES

Animals

This study was approved by the Animal Research Committee of Zhejiang University, School of Medicine. A total of 28 male Sprague–Dawley rats (250–280 g, grade II, Certificate No. SCXK2003-0001, Experimental Animal Center, Zhejiang Academy of Medical Science, Hangzhou, China) were maintained in individual cages with a 12-h light/dark cycle. All experiments were carried out in accordance with the ethical guidelines of the Zhejiang University Animal Experimentation Committee and were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Efforts were made to minimize the number of animals used in this study and to minimize their suffering. Water and food were given *ad libitum*.

MRI scan

The MRI scan was taken under chloral hydrate anesthesia (400 mg/kg, i.p.; C8383, Sigma, USA) using a 3.0 T scanner system (Signa, Excite HD, GE, USA). The rats were placed in a rodent coil for the duration of the scan. After scout images were obtained, a 3D FSPGR T1-weighted image (repetition time=8.7 ms; echo time=4.3 ms; inversion time=450 ms; 256×256 matrix; slice thickness=0.3 mm; field of view=60 mm; taking 5 min 11 s) of the rat brain was acquired.

Surgery

Surgery for electroencephalographic (EEG) recordings was performed under chloral hydrate anesthesia. Screw electrodes were inserted into the skull bilaterally over the frontal cortex as previously described (Gao et al., 2009; Wang et al., 2008). After surgery, rats were allowed 2 weeks of recovery.

Pilocarpine protocol and continuous EEG monitoring

After 2 weeks of recovery and a baseline microPET scan, rats were injected with pilocarpine (360 mg/kg i.p.; P6503, Sigma, USA) to induce SE. Methscopolamine bromide (1 mg/kg i.p.; S8502, Sigma) was injected 30 min prior to the administration of pilocarpine to reduce peripheral effects. If the rat did not develop SE within 45 min, an additional dose of pilocarpine (110 mg/kg) was given. Of the 28 rats, 23 did not require further injections. All rats received a single dose of diazepam (20 mg/kg i.p.; D0899, Sigma) 120 min after SE onset. Nine rats died after SE onset and were therefore excluded from the study.

An EEG monitoring system (RM-6240B, Chengyi, China) with digital cameras (GZ-MG330, JVC, Japan) was used to record SRS and synchronous EEG activity. After pilocarpine injection, EEG monitoring began. The EEG was analyzed to detect SRS as previously described (Goffin et al., 2007). Electrographic seizure was defined as a discharge with a frequency >5 Hz, an amplitude >2× baseline and a duration >10 s. When an electrographic seizure was detected, the behavioral severity was determined from the video recordings and classified according to the scoring scale defined by Veliskova (2006). Animals that developed SRS were defined as the SRS group, and those that did not develop SRS were defined as the non-SRS group.

MicroPET scan

Every rat received microPET scans at baseline and the early, latent and chronic phases. The baseline scan was performed 2 weeks after surgery, while the remaining scans were performed at 2 days, 7 days, and 42 days after SE onset, respectively.

The PET detector used for this study was microPET R4 (Concorde Microsystems, USA), which consists of a 15-cm diameter ring of 96 position-sensitive γ-ray scintillation detectors, providing a 10.8-cm transaxial and a 7.8-cm axial field of view, with image resolution <1.8 mm. The transaxial image planes were separated by 1.21 mm 18F-FDG, with a specific activity of 500 Ci/mmol, was administered i.v. through the dorsal penile vein, after which the rats were returned to their home cage in a room with minimal ambient noise for the duration of the uptake period of 40 min. Subsequently, animals were placed in the microPET scanner under isoflurane gas anesthesia (5% induction and 1.5% for maintenance). A 15-min static acquisition was performed in 3D mode. Data were collected in list mode and reconstructed by a maximum-a-posteriori probability algorithm with a pixel size of $0.4\times0.4\times1.2$ mm^3.

MRI and PET data analysis

The image data acquired from microPET were displayed and analyzed by IDL (ver. 6.2, Research Systems, USA) and ASIPro VM (ver. 6.0.5.0, Concorde Microsystems Inc.) software. The PET and MRI images were co-registered using ASIPro VM software as previously described (Gao et al., 2009). Axial (coronal) MRI slices (n=17) of a rat brain were used to assign regions of interest (ROIs) per slice according to the rat brain atlas (Paxinos and Watson, 2005). A total of 11 brain areas were evaluated, including the prefrontal, sensorimotor, visual, auditory, entorhinal, piriform cortices, the striatum, thalamus, hippocampus, cerebellum and the pons (Figs. 1 and 2). In order to compensate for global brain activity, the regional glucose metabolic rate (CMRglc) values of each ROI were normalized (nCMRglc) by dividing by the glucose metabolic rate of the pons (Kornblum et al., 2000). The nCMRglc values are expressed as a percentage (%) [FDG uptake=(study ROI/pons ROI)×100] (Table 1). The pons was chosen for normalization because its metabolic rate is known to be unaffected by pilocarpine-induced seizures in any of the three phases studied (Arida et al., 2003; Dubé et al., 2001; Fernandes et al., 1999; Handforth and Treiman, 1995; Kornblum et al., 2000). Other methods, such as the percentage of injected dose, are considered less reliable measures of the availability of FDG to the brain, as extravasation of the tracer at the site of injection may occur (Kornblum et al., 2000). Because of the large individual variability of baseline levels in each ROI, the changes of nCMRglc values in all three phases are expressed in percent relative to baseline (100%) in ROIs: [$\text{Changes}_{x-\text{baseline}}=(\text{nCMRglc}_x-\text{nCMRglc}_{\text{baseline}})/\text{nCMRglc}_{\text{baseline}}\times100$] (x=early or latent or chronic, Fig. 3).

Statistical analyses

Values are presented as mean±SEM. Statistical analyses were performed using SPSS for Windows 12.0. A multiple comparison using the Bonferroni correction was applied in order to compare nCMRglc values among the different phases in either group ($P<0.05$ was considered significant). A comparison between SRS