In Vivo Imaging of mGluR5 Changes during Epileptogenesis Using \([11C]\)ABP688 PET in Pilocarpine-Induced Epilepsy Rat Model

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Abstract

Introduction: Metabotropic glutamate receptor 5 (mGluR5) that regulates glutamatergic neurotransmission contributes to pathophysiology of epilepsy. In this study, we monitored the changes of mGluR5 in vivo using \([11C]\)ABP688 PET during the epileptogenesis in a pilocarpine-induced epilepsy rat model.

Methods: In vivo mGluR5 images were acquired using \([11C]\)ABP688 microPET/CT in pilocarpine-induced chronic epilepsy rat models and controls. We also acquired microPET/CT at acute, subacute as well as chronic periods after status epilepticus. Non-displaceable binding potential (BPND) of \([11C]\)ABP688 was calculated using simplified reference tissue model in a voxel-based manner. mGluR5 BPND of the rat brains of epilepsy models and controls were compared.

Results: Status epilepticus developed after pilocarpine administration and was followed by recurrent spontaneous seizures for more than 3 weeks. In chronic epilepsy rat model, BPND in hippocampus and amygdala was reduced on a voxel-based analysis. Temporal changes of mGluR5 BPND was also found. In acute period after status epilepticus, mGluR5 BPND was reduced in the whole brain. BPND of caudate-putamen was restored in subacute period, while BPND of the rest of the brain was still lower. In chronic period, global BPND was normalized except in hippocampus and amygdala.

Conclusions: In vivo imaging of mGluR5 using \([11C]\)ABP688 microPET/CT could successfully reveal the regional changes of mGluR5 binding potential of the rat brain in a pilocarpine-induced epilepsy model. The temporal and spatial changes in mGluR5 availability suggest \([11C]\)ABP688 PET imaging in epilepsy provide abnormal glutamatergic network during epileptogenesis.

Introduction

Glutamate mediated neurotransmission is important in the pathogenesis of epilepsy. Metabotropic glutamate receptors (mGluRs) play a role in the initiation of epileptic discharge and propagation [1–3]. In particular, group I mGluRs (mGluR1 and mGluR5) are involved in making abnormal synaptic plasticity which induce long-lasting depolarization and activate neurons to persistently hyperexcitable state [3–5]. Therefore, there has been a growing interest in mGluR-mediated neuronal transformation to develop spontaneous recurrent seizures, which contributes crucially to epileptogenesis.

The abnormalities of mGluR expression in epilepsy were found both in human and animal studies. In focal cortical dysplasia, strong immunoreactivity of group I mGluRs in dysplastic neuronal cells suggested possible contribution of mGluRs to epileptogenesis [6]. In human temporal lobe epilepsy (TLE), Blümcke, et al. reported up-regulated mGluR1 though mGluR5 did not show any significant change [7], however, Notenboom, et al. showed up-regulation of mGluR5 in TLE patients, particularly in hippocampal non-sclerosis groups [8]. In pilocarpine-induced epilepsy animal models, mGluR5 protein expression decreased in the hippocampus, and mGluR-mediated hippocampal long term depression (LTD) was reduced [9,10]. Though mGluR expression results were inconsistent in epilepsy studies, mGluRs were
proposed to be an important molecular target for developing new antiepileptic drugs [2,11].

Recently, a positron emission tomography (PET) tracer, 3-(6-methyl-pyridin-2-yl-ethyl)-cyclohex-2-enone-O-11C-methyl-oxime ([11C]ABP688), was developed as a highly selective antagonist of mGluR5 [12]. As well as in animal models, using [11C]ABP688 PET, mGluR5 status was examined in patients with major depression or in smokers and ex-smokers [13,14]. Furthermore, the studies to measure mGluR5 receptor availability based on the tracer kinetics for reversible ligands were performed in humans and rodents [15,16].

As mGluR5 is supposed to be involved in epileptogenesis, we examined the localized abnormalities of mGluR5 in a chronic epilepsy rat model using [11C]ABP688 PET. We also studied temporal patterns of mGluR5 availability after status epilepticus and tried to understand the abnormal glutamatergic networks in epileptogenesis using a pilocarpine-induced epilepsy model.

Materials and Methods

Establishment of epilepsy rat model

Twenty-two adult male Sprague-Dawley (SD) rats (7 weeks old; Koatech, Seoul, Korea), weighing 180–200 g were used as controls (n = 7) and models (n = 15). They were kept at standard laboratory condition (22–24°C, 12 hour light and dark cycle) with free access to water and standard feed. All the experimental procedures were approved by Institutional Animal Care and Use Committee at Seoul National University Hospital (IACUC Number 13-0224).

Rats were pretreated with lithium chloride (127 mg/kg, i.p., Sigma, St. Louis, MO) and methylscopolamine-bromide (1 mg/kg, i.p. Sigma) 24 hours and 30 min before pilocarpine administration, respectively. Pilocarpine hydrochloride (30 mg/kg, i.p., Sigma) was injected to trigger status epilepticus. Repeated doses of pilocarpine hydrochloride for 10 mg/kg were then administered every 30 min until stage 4 seizures developed according to the Racine scale [17]. The control group received lithium chloride, methylscopolamine-bromide and saline (sham treatment) instead of pilocarpine. Status epilepticus was defined as continuous generalized seizures with stage 4 or 5 according to Racine scale without normal behavior between seizures. Diazepam (10 mg/kg, i.p. Samjin, Seoul, Korea) was injected 60 min after the onset of status epilepticus to terminate seizure activity. Repeated diazepam (5 mg/kg) was administered unless status epilepticus was terminated to reduce mortality. After cessation of status epilepticus, rats were treated with supplementary moistened and crushed pellets soaked in Gatorade on the cage floor and given 5 mL i.p. injection of 0.9% saline for hydration in the rats unable to drink. Among 15 rats of experimental group, 11 rats survived in acute and subacute periods to yield 5 rats for PET examination in chronic period.

The model rats in chronic period were monitored using a video recorder (12 h/day, for 2 days) to evaluate spontaneous recurrent seizure. Spontaneous recurrent seizures were observed in all chronic epilepsy rats.

PET experimental design

PET scans were acquired in chronic epilepsy rats and controls. Chronic period was more than 3 weeks after status epilepticus (median 44 d, range 34 d–59 d). In 7 control rats, PET scans were acquired median 33 d (range 18 d–52 d) after sham treatment.

To find temporal changes after status epilepticus, PET scans were acquired at acute and subacute periods in experimental group: acute period was defined as 1 day after status epilepticus, and subacute period defined as 7 days after status epilepticus. Because of general condition of rats, all the rats were not repetitively scanned for each period. PET scans were successfully obtained for 4 rats in acute period, 4 rats in subacute period and 5 rats in chronic period after status epilepticus. PET experiments in this study were summarized in Figure 1.

Synthesis of [11C]ABP688

ABP688 and desmethyl-ABP688 were prepared according to previously reported method [12] and all synthesized products were confirmed by 1H-NMR and mass spectroscopy. All other chemical reagents were used as purchased without any purification.

[11C]ABP688 synthesis was performed in a closed and lead-shielded hot cell by loop method [18]. Briefly, 60% sodium hydride (5 mg, 0.12 mmol) was added to a solution of desmethyl-ABP688 (1 mg, 4.42 μmol) in anhydrous N,N-dimethylformamide (200 μL) and then the mixture was filtered. The resulting solution was loaded into stainless steel loop of injection port of HPLC. [11C]CH3I produced from [11C]CO2 using TRACERlab FX C Pro module (GE Medical Systems, Sweden) was passed through the loop and reacted with precursor at room temperature for 3 min. The product was purified by preparative HPLC (Xterra preparative column RP8, 10 μm, 10×250 mm, Waters; mobile phase, EtOH:10 mM NaH2PO4 [50:50], isocratic; flow rate, 4 mL/min). The purified [11C]ABP688 (retention time of 10–

| Models (n=15) | Acute Period (1 day) | Subacute Period (7 days) | Chronic Period (> 3 weeks) |
|-------------|----------------------|--------------------------|---------------------------|
| n = 4       |                      | n = 1                    | n = 1                     |
| Unexpected death (n = 4) |                    |                          |                          |

Figure 1. Numbers of PET scans at each time point. After status epilepticus induced by pilocarpine administration, four rats were scanned in acute period. Three rats were scanned in subacute period without PET acquisition in acute period and four rats were scanned in chronic period after status epilepticus. Among the four rats scanned in acute period, a rat was scanned in subacute period and among the three rats scanned in subacute period, a rat was scanned in chronic period, repetitively (dotted line).

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11 min) was passed through a sterile Millex FG and collected in a sterile vial. The final product was diluted with sterile saline for injection.

The radiochemical purity and specific activity of $^{[11C]}$ABP688 were determined by the analytical HPLC (Sierra analytical column RP18, 3.5 μm, 4.6×100 mm, Waters; mobile phase, acetonitrile:water [50:50], isocratic; flow rate, 1.5 mL/min). The retention time of $^{[11C]}$ABP688 in analytical HPLC was 4–4.5 min.

$[^{11}C]ABP688$ PET acquisition

PET scans were performed on a dedicated microPET/CT scanner (eXplore VISTA, GE Healthcare). All animals were anesthetized and maintained with 1% isoflurane at 1 L/min oxygen flow and placed on the prone position under a scanner. Rats received an intravenous bolus injection (0.2–0.5 mL/rat) of $^{[11C]}$ABP688 (7.0–17.1 MBq/100 g) and list-mode data were acquired for 60 min with the energy window 400–700 keV. These list mode data were framed into a dynamic sequence of $6 \times 30$ s, $7 \times 60$ s, and $5 \times 600$ s frames. The images were reconstructed by a 3-dimensional ordered-subsets expectation maximum (OSEM) algorithm with attenuation, random and scatter correction. The voxel size was 0.3075×0.3075×0.3775 mm.

Image preprocessing, kinetic modeling and parametric mapping

Individual summed PET images were obtained from all rats (0–60 min summed images) followed by manual cropping of the necessary parts to include the entire brain. For the seven control rats, images were spatially normalized to a representative brain. A voxel-based average brain image was constructed using nonlinear warping method after linear affine transformation. All transformations were performed using BioImage Suite software package (www.bioimagesuite.org, Yale University) and registration was visually confirmed. In order to obtain $^{[11C]}$ABP688 PET template, the mean image was co-registered with a standard MRI T2 template using linear affine transformation [19]. All the PET images including experimental and control groups were spatially normalized to the $^{[11C]}$ABP688 PET template.

Regional time-activity curves (TACs) were calculated using the pre-defined volumes of interest (VOIs) on the rat template consisting of caudate-putamen, hippocampus, amygdala, frontal cortex, and cerebellum [19]. For quantitative analysis, we used kinetic modeling analysis and generated parametric images of $^{[11C]}$ABP688 PET. Non-displaceable binding potential ($BP_{ND}$) was used to evaluate receptor availability. $BP_{ND}$ was calculated by the simplified reference tissue model (SRTM) [20,21] and the cerebellum was used as a reference region for the mGluR5 quantification [16].

Kinetic analyses and voxel-based $BP_{ND}$ mapping were performed using MATLAB (MathWorks, Natick, MA) and C-based programs of Turku PET center (http://www.turkupetcentre.fi, Turku PET Centre, Finland). Parametric $BP_{ND}$ maps were smoothed with a Gaussian filter of 1.2 mm full width at half maximum (FWHM).

Voxelwise analysis of parametric maps

The $BP_{ND}$ parametric maps of the rats in chronic period were compared with those of control rats on a voxel basis. Two-sample t-test was performed between two groups using statistical parametric mapping software package (SPM2; University College London, London, England). Uncorrected values of $P<0.001$ were set as the significance threshold and an extent threshold of 30 contiguous voxels was applied.

In order to evaluate the significance of changes in $BP_{ND}$ of chronic epilepsy models, and to re-confirm the results of the voxel-based analysis, a post-hoc VOI analysis was performed using nonparametric Mann-Whitney test in the significant clusters with decreased $BP_{ND}$.

Temporal changes of mGluR5 $BP_{ND}$

We obtained mGluR5 $BP_{ND}$ of predefined VOIs including caudate-putamen, hippocampus and amygdala in models in different periods and controls. Using spatially normalized $BP_{ND}$ maps and the VOIs, regional $BP_{ND}$ was obtained in models and controls. Regional $BP_{ND}$ in epilepsy models in acute, subacute, and chronic periods were compared with that in controls.

Statistical analysis

Data were expressed as mean ± SD. To test for differences in the regional $BP_{ND}$ obtained from predefined VOIs, nonparametric Mann-Whitney test was performed between models in different periods and controls. We also performed Mann-Whitney test in post-hoc voxel-based analysis, i.e. comparison of $BP_{ND}$ in the significant clusters between models in chronic period and controls. The statistical analyses were performed using SPSS software (version 18; SPSS Inc., Chicago, IL).

Results

Time-activity curve for $^{[11C]}$ABP688

Figure 2 shows representative time-activity curves (TACs) of controls and models in acute, subacute, and chronic periods. TACs were drawn for 1) caudate-putamen, 2) hippocampus, 3) frontal cortex, 4) amygdala and 5) cerebellum. In all models and controls, high mGluR5 bindings were observed in caudate-putamen, hippocampus and lower bindings were seen in the cerebellum. Temporal changes of TACs in pilocarpine-induced epilepsy model revealed globally decreased activity in acute period compared to subacute and chronic periods after status epilepticus as well as controls. Note that standardized uptake value (SUV) is defined as the tissue concentration of $^{[11C]}$ABP688 in the VOIs (MBq/mL) divided by the activity injected per gram of body weight (MBq/g).

Chronic epilepsy models vs. controls

Using predefined VOIs, mGluR5 $BP_{ND}$ of each brain region of chronic models was calculated and compared with that of controls. There was no significant difference in regional mGluR5 $BP_{ND}$ between chronic epilepsy models and controls for the above four regions and amygdala on VOI analysis. $BP_{ND}$ of caudate-putamen was $2.08\pm0.18$ and $2.13\pm0.45$, $BP_{ND}$ of hippocampus was $1.63\pm0.18$ and $1.52\pm0.43$ and $BP_{ND}$ of amygdala was $1.33\pm0.15$ and $1.19\pm0.37$, for controls and chronic models, respectively.

Voxel-based analysis revealed the areas showing the significant difference in $BP_{ND}$ between chronic epilepsy and controls. As is shown in Figure 3A, four clusters mainly involving the part of bilateral dorsal hippocampus and amygdala showed lower mGluR5 $BP_{ND}$ in chronic epilepsy models than controls. Increased regional $BP_{ND}$ was not found in the model rats.

Post-hoc analysis was performed on two clusters mainly in the left dorsal hippocampus and left amygdala among the four clusters because of relatively higher T-scores and larger cluster size (Figure 3B). $BP_{ND}$ of the cluster 1, a VOI on dorsal hippocampus, was significantly lower in the models in chronic period than that of
controls ($U = 5$, $p = 0.048$), and there was a trend for decreased BPND in chronic model in cluster 2, a VOI on amygdala ($U = 6$, $p = 0.06$).

**Temporal changes of mGluR5 BPND in acute and subacute periods**

mGluR5 BPND of caudate-putamen decreased in acute period ($1.53 \pm 0.26$ compared to controls ($2.13 \pm 0.45$) ($U = 0$, $p < 0.01$ for acute period vs. control). In the hippocampus, mGluR5 BPND of epilepsy models decreased in acute ($1.11 \pm 0.20$) and subacute ($1.20 \pm 0.18$) periods compared to controls ($1.63 \pm 0.18$) ($U = 0$, $p < 0.01$ for acute period vs. control and for subacute period vs. control). Similarly to hippocampus, mGluR5 BPND in the amygdala was decreased in acute ($0.94 \pm 0.18$) and subacute ($0.82 \pm 0.10$) periods compared to controls ($1.33 \pm 0.15$) ($U = 1$, $p < 0.05$ for acute period vs. control and $U = 0$, $p < 0.01$ for subacute period vs. control) (Figure 4).

Parametric images of mGluR5 BPND were generated using SRTM. As shown in Figure 4D, in acute period, BPND decreased globally in the whole brain, including the caudate-putamen and hippocampus. The BPND of caudate-putamen recovered in subacute period though it was still low in hippocampus and amygdala. In chronic period, regional mGluR5 BPND in the hippocampus and amygdala VOIs (not confined to significant voxels within these regions, in that ‘significant’ means the clusters 1 or 2 in Figure 3B) recovered in comparison to that of subacute period.

**Discussion**

This study analyzed mGluR5 availability of the rat brain in a pilocarpine-induced epilepsy model using [$^{11}$C]ABP688 microPET/CT. Using SRTM, we measured mGluR5 availability by BP$_{ND}$. In chronic epilepsy models which are characterized by spontaneous recurrent seizures, voxel-based analysis and posthoc comparison revealed that BP$_{ND}$ in the dorsal hippocampus and amygdala was significantly lower than those of controls. We also found the temporal pattern of mGluR5 BP$_{ND}$ change after status epilepticus, that mGluR5 BP$_{ND}$ decreased in the rat brain of epilepsy model globally in acute period after status epilepticus. In subacute period, regional BP$_{ND}$ in caudate-putamen was restored, though BP$_{ND}$ in hippocampus and amygdala was still lower. Decreased mGluR5 BP$_{ND}$ in hippocampus and amygdala sustained in chronic period in this rat model of pilocarpine-induced medial temporal lobe epilepsy.

mGluR5 expression has been studied using surgical and postmortem specimens in patients as well as in animal models [8–10]. However, the findings of these biopsy studies could not be obtained preoperatively. [$^{11}$C]ABP688 PET enabled quantitative assessment of receptor availability and its serial noninvasive monitoring [13–15]. Reminded by the use of [$^{11}$C]ABP688 PET.
to examine mGluR5 availability in patients, we observed the spatial distribution of the changes in mGluR5 availability and its temporal progress during epileptogenesis in the rat model of a chronic temporal lobe epilepsy.

In this study, the changes of mGluR5 availability were partly consistent with previous pathologic studies using in vitro techniques in the same pilocarpine-induced epilepsy model. In previous pathologic studies, pilocarpine-induced chronic epileptic rats revealed reduced mGluR5 expression in the hippocampus [9]. Furthermore, at 24 h after status epilepticus, mGluR5 expression decreased in amygdala, piriform and entorhinal cortices [10]. Our study revealed reduced receptor availability in bilateral hippocampi in chronic epilepsy rat models. mGluR5 receptor availability was reduced in the brain globally at 24 h after status epilepticus and this abnormality persisted in the amygdala and dorsal hippocampus in subacute and chronic periods, though the receptor availability was restored mostly in other areas in chronic period.

Pilocarpine-induced status epilepticus is characterized by generalized tonic-clonic seizures. The rats go into a seizure-free period during 2–3 weeks, followed by chronic period with spontaneous seizure activity. Decreased mGluR5 $BP_{ND}$ in acute period after status epilepticus could be associated with postictal status, while that in subacute period might be independent to acute seizure activity because the rats were seizure-free. Thus, temporal changes in mGluR5 $BP_{ND}$ implied gradual molecular changes.

Figure 3. Spatial distribution of changes of mGluR5 $BP_{ND}$ in chronic epilepsy model rats on $[^{11}C]ABP688$ PET. Compared with controls, $BP_{ND}$ decreased in the bilateral amygdala and dorsal hippocampi (A: p < 0.001, uncorrected). (B) Nonparametric post-hoc VOI-based analysis performed on two clusters of decreased $BP_{ND}$ on voxel-based analysis. $BP_{ND}$ of cluster 1 (C1) on dorsal hippocampus in chronic epilepsy models decreased significantly compared to controls (p = 0.048). There was a trend for decrease in $BP_{ND}$ of cluster 2 (C2) on amygdala in chronic epilepsy models compared to controls (p = 0.06).

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changes during epileptogenesis rather than epileptic seizure activity itself.

In acute period after status epilepticus, overactivation of glutamatergic neuron might lead to reduced availability of mGluR5 in the entire brain. Glutamate release occupies the postsynaptic receptors after pilocarpine-induced long-lasting status epilepticus [22]. Thus, mGluR5 availability would become globally decreased in the acute period. A previous study reported increased glutamate level induced by N-acetylcysteine reduced mGluR5 $B_P_{ND}$, though ABP688 is allosteric modulator [23]. In this context, excessive glutamate release during seizure could contribute to the reduction of mGluR5 $B_P_{ND}$ in acute period.

Figure 4. mGluR5 binding potential ($B_P_{ND}$) decreased in epilepsy models according to time periods after status epilepticus. (A–C) $B_P_{ND}$ decreased globally in acute period after status epilepticus. In subacute period, mGluR5 $B_P_{ND}$ recovered in caudate-putamen, while decreased yet in hippocampus and amygdala, and in chronic period, there was no significant difference in $B_P_{ND}$ between model rats and controls using VOI-based analysis. (D) Representative $B_P_{ND}$ parametric maps for model rats and controls were consistent with temporal patterns of VOI-based analysis. Note that $B_P_{ND}$ was visually normalized except in hippocampus and amygdala in chronic period, which corresponded to the voxel-based analysis (arrowheads: hippocampus, arrows: amygdala). Error bars represent standard errors of the mean (SEM). * $p<0.05$; ** $p<0.01$. doi:10.1371/journal.pone.0092765.g004
mGluR5 availability measured on $[^{11}C]ABP688$ PET has a complex relation with mGluR5 expression of the neurons, in that various factors could contribute to the decrease of mGluR5 $BP_{ND}$ in the epileptic rat brain. In the study by Kirschstein et al., the decrease of mGluR5 expression was mainly caused by down-regulation of receptor molecules in the neurons but they reported that the neuronal cell loss also partially contributed to this decrease [9]. Magnetic resonance imaging (MRI) studies also showed progressively decreasing hippocampal volume in the pilocarpine-induced epilepsy model [24,25]. Thus, both volume decrease and down-regulation should be considered when explaining the low $[^{11}C]ABP688$ binding in the hippocampus and amygdala in the chronic period observed in our investigation. By the way, as we found that decreased $BP_{ND}$ of mGluR5 in acute period recovered to normal already in subacute period globally in the brain, which was different from MRI findings of global progressive volume reduction [25]. In chronic period, mGluR5 $BP_{ND}$ recovered in the entire brain except for the focal areas of bilateral amygdala and dorsal hippocampus. We speculate that down-regulation of mGluR5 receptor functional activity explains mostly the decreased $BP_{ND}$ in hippocampus and amygdala in the chronic period of pilocarpine-induced epileptic rats. To elucidate this, further study to examine serial sequential changes of mGluR5 availability in the same individual animals during the epileptogenesis after pilocarpine-induction of epilepsy is warranted. Simultaneous $[^{11}C]ABP688$ PET and magnetic resonance imaging as well as postmortem histologic studies might provide comprehensive epileptogenic changes in receptors and neuronal density.

One of the important issues of cross-sectional molecular studies in epilepsy is whether it underlies the epileptogenesis or result of epileptogenic processes [26]. In this study, the cause of mGluR5 $BP_{ND}$ reduction is unknown and could be a result of epileptogenesis. One of the possible mechanisms of mGluR5 changes was an intrinsic antiepileptic response induced by status epilepticus. As mGluR5 antagonist reduces excitability, the reduction of mGluR5 availability in chronic pilocarpine-induced epilepsy may represent an endogenous antiepileptic effort [27,28]. This interpretation depends on the previous suggestion of feedback regulation of excitability related to neuronal homeostasis [29,30]. Alternatively, neuronal network abnormality due to mGluR5 signal reduction would work in chronic period of pilocarpine-induced epilepsy. Decreased mGluR5 expression induced the reduction of long term depression, thus causing the abnormal activities of the neuronal network in the epileptic brain [9]. In genetic autism models, mGluR5 decrease caused network abnormalities [31], where mGluR5 abnormality underlay the pathogenesis of autistic features. Though the causal relationship between mGluR5 changes and epileptogenesis is still unclear, the spatial and temporal changes in mGluR5 using $[^{11}C]ABP688$ PET provided an essential macroscopic view of functional features of glutamatergic synapses of epileptic rat brain associated with epileptogenesis.

This study has some limitations. We studied the temporal changes in mGluR5 $BP_{ND}$ after status epilepticus, however, the controls were only assessed at chronic periods, not at all the time points. Repeated anesthesia and imaging was difficult for seriously ill epileptic rats, though. Temporal patterns of mGluR5 $BP_{ND}$ changes were not exactly paired and individual variation between unpaired data would have obliterated the subtle differences between groups or changes within groups. Despite this limitation, however, the results showed prominent temporal changes of mGluR5 $BP_{ND}$ through the acute and subacute periods and finally in chronic period. We used $[^{11}C]$-labeled compound, thus, relatively short physical half-life compared with $[^{18}F]$-labeled compound was disadvantage in clinical application. However, compared to recently developed $[^{18}F]$FPED, $[^{11}C]ABP688$ has advantages in well-established kinetics and receptor-ligand properties [13,16]. Several mGluR5 targeted tracers including $[^{18}F]$FPED could be used to estimate mGluR5 availability in the future [32]. In our study, because the main purpose was to evaluate $[^{11}C]ABP688$ mGluR5 availability in epilepsy models, neither EEG monitoring of the rats nor frequency of spontaneous recurrent seizures was documented. mGluR5 imaging correlated with clinical features may provide functional grading and classification for TLE.

$[^{11}C]ABP688$ PET promises to enable us to evaluate the mGluR5 changes during epileptogenesis and pathogenesis of neurological diseases. Because $[^{11}C]ABP688$ PET was already used in patients with neurological and psychiatric disorders [14,15], we propose that $[^{11}C]ABP688$ PET be used in epilepsy patients hopefully to find seizure focus as well as to evaluate the progress of pathophysiology of epilepsy. Being reminded by the report that mGluR5 expression did not decrease but increase in the hippocampus in medial TLE patients [8], $[^{11}C]ABP688$ PET findings should be interpreted comprehensively because they reflected the mixture of primary pathology and compensatory changes of the human brain to epileptogenesis. $[^{11}C]ABP688$ PET might enlighten us in classifying seizure disorders in a refined fashion in terms of glutamatergic neurotransmission during pathophysiologic progress of intractable medial temporal lobe epilepsy in human as well as in rat models.

**Conclusion**

We showed the *in vivo* imaging of mGluR5 in pilocarpine-induced epilepsy rat models using $[^{11}C]ABP688$ microPET/CT. PET imaging in the present study revealed mGluR5 changes during epileptogenesis in the rat models, and also could localize abnormal mGluR5 availability associated with chronic period of epilepsy. The temporal and spatial changes of mGluR5 availability may provide abnormal glutamatergic network during epileptogenesis.

**Author Contributions**

Concept and designed the experiments: YKK DSL. Performed the experiments: HC SWO HJI DWH YSL. Analyzed the data: HC YKK SWO HJI HK. Contributed reagents/materials/analysis tools: BL YSL JMJ. Wrote the paper: HYC YKK DSL SWO HJI DWH BL EEEK JKC.

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