

In vivo activation of endocannabinoid system in temporal lobe epilepsy with hippocampal sclerosis

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The endocannabinoid system modulates neuronal excitability, protects neurons against hyperexcitability and is involved in epileptogenesis in animal models of mesial temporal lobe epilepsy with hippocampal sclerosis. We performed *in vivo* positron emission tomography imaging of the type 1 cannabinoid receptor in patients with mesial temporal lobe epilepsy with hippocampal sclerosis. Twelve patients with refractory mesial temporal lobe epilepsy due to hippocampal sclerosis received a [¹⁸F]MK-9470 scan to assess type 1 cannabinoid receptor availability *in vivo*. Parametric modified standard uptake values were used as quantitative measure of type 1 cannabinoid receptor availability and images were spatially normalized to standard space. Voxel-based analysis was performed comparing patients with hippocampal sclerosis to controls and correlations between type 1 cannabinoid receptor status and seizure characteristics were done using volumes of interest. Type 1 cannabinoid receptor positron emission tomography was co-registered with subtraction ictal single photon emission computed tomography co-registered to magnetic resonance imaging of a complex partial seizure ($n = 9$). An increased type 1 cannabinoid receptor availability in the ipsilateral temporal lobe was observed, which correlated negatively with the latency since last seizure before scanning and positively to the number of seizures in the month before scanning. A decreased type 1 cannabinoid receptor availability was present in the superior insular cortex, ipsilateral more than contralateral. The ipsilateral insular region displayed a mild ictal hyperperfusion in the transition zone of subtraction ictal single photon emission computed tomography co-registered to magnetic resonance imaging temporal lobe hyperperfusion-frontal lobe hypoperfusion during complex partial seizures. Type 1 cannabinoid receptor availability showed opposite changes in different brain regions that are involved during complex partial seizures in refractory mesial temporal lobe epilepsy with hippocampal sclerosis. The increase in type 1 cannabinoid receptor availability at the seizure onset zone might be a protective mechanism of neurons against hyperexcitability and seizure activity, or contribute to the process of epileptogenesis, or both. The decreased type 1 cannabinoid receptor availability in the insula may play a role in surround inhibition and prevention of seizure propagation.

Keywords: type 1 cannabinoid receptor; hippocampal sclerosis; epilepsy; [¹⁸F]MK-9470; positron emission tomography

Abbreviations: CB1R = type 1 cannabinoid receptor; SISCOM = subtraction ictal single photon emission computed tomography co-registered to MRI

Introduction

Epilepsy affects ~3% of the population during their lifetime (Hauser, 1975) and can be successfully treated using anti-epileptic drugs in ~70% of patients. New potent anti-epileptic drugs are therefore still needed. A small group of patients with refractory partial epilepsy can be treated with surgical removal of the epileptogenic zone. In order to perform surgery, the seizure onset zone needs to be identified clearly taking results of clinical seizure semiology, ictal and interictal EEG, dedicated MRI, subtraction ictal single photon emission computed tomography co-registered to MRI (SISCOM), 2-[¹⁸F]-fluoro-2-deoxy-D-glucose PET and neuropsychological testing into account (Goffin *et al.*, 2008). Despite this multitude of tests, it is not possible to reliably delineate the seizure onset zone in some patients. To help these patients, new diagnostic tools to improve the detection of the seizure onset zone are important.

Type 1 cannabinoid receptors (CB1Rs) are expressed in high concentrations in the CNS, where they are not only present on neurons, but also in lower concentrations on microglia, astrocytes and oligodendrocytes (Di Marzo *et al.*, 1998). CB1Rs, located mainly on presynaptic nerve terminals, modulate neuronal excitability through suppression of the release of other neurotransmitters, for example glutamate, GABA and dopamine (Wilson and Nicoll, 2002).

The endocannabinoid system provides 'on-demand' protection against hyperexcitability and acute seizures and is neuroprotective against excitotoxicity via a CB1R-dependent mechanism (Marsicano *et al.*, 2003). In animal models of mesial temporal lobe epilepsy due to hippocampal sclerosis, CB1R-agonists have a strong anti-epileptic effect (Wallace *et al.*, 2003). In humans, the effect of CB1R-agonists in epilepsy is less clear and not well studied. Consroe and colleagues (1998) suggested that cannabis may have anti-epileptic effects, but others found it ineffective or proconvulsant (Gordon and Devinsky, 2001; Lutz, 2004).

Mesial temporal lobe epilepsy due to hippocampal sclerosis is often preceded by an initial precipitating injury (Mathern *et al.*, 1995), such as a prolonged febrile convulsion, cerebral trauma or meningoencephalitis. The time between initial precipitating injury and onset of habitual epileptic seizures is called the silent period or latent interval. Epileptogenesis refers to a process in which an initial brain-damaging insult triggers a cascade of molecular and cellular changes that eventually lead to occurrence of spontaneous seizures. In animal models of mesial temporal lobe epilepsy due to hippocampal sclerosis, the endocannabinoid system has been implicated in the process of epileptogenesis (Goffin *et al.*, 2009), and early administration of CB1R-antagonists was anti-epileptogenic and prevented the development of epilepsy (Chen *et al.*, 2007).

In patients with mesial temporal lobe epilepsy due to hippocampal sclerosis, only *in vitro* studies have been performed to date, demonstrating a decreased level of CB1R messenger RNA in the sclerotic hippocampus (Ludanyi *et al.*, 2008).

In this article, we have used [¹⁸F]MK-9470, a radioligand with high affinity and specificity for the CB1R (Burns *et al.*, 2007), and PET imaging in a cross-sectional study. The aim was to assess

CB1R availability in patients with mesial temporal lobe epilepsy due to hippocampal sclerosis *in vivo*, to correlate CB1R availability with clinical data and to determine the potential role of CB1R imaging as a supplementary tool in identifying the seizure onset zone during presurgical evaluation.

Materials and methods

Patient characteristics

A total of 12 patients with refractory mesial temporal lobe epilepsy due to hippocampal sclerosis (seven males, five females; age range 28–70 years) were included. The seizure onset zone was defined using a combination of seizure semiology, interictal and ictal EEG, optimized MRI, ictal and interictal brain perfusion single photon emission computed tomography imaging and SISCOM analysis ($n = 9$) (Table 1).

A population of 50 Caucasian volunteers (25 female, 25 male; age range 18–69 years) from a previous study was available as the control group (Van Laere *et al.*, 2008).

The study was approved by the local Ethics Committee and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects prior to the study.

Radiotracer characteristics and preparation

[¹⁸F]MK-9470 (Merck Research Laboratories, West Point, PA, USA) is an inverse agonist with high selectivity and specificity for the human CB1R (Burns *et al.*, 2007). The precursor for tracer synthesis was obtained from MRL and labelling was performed on-site using ¹⁸F-ethylbromide. The final product was obtained after high-performance liquid chromatography separation and had a radiochemical purity >95%. Specific activity was higher than 200 GBq/μmol. The tracer was administered in a sterile solution of 5 mM sodium acetate buffer, pH 5.5 containing 6% ethanol.

Imaging procedure

All patients were fasted for at least 4 h prior to PET imaging. Subjects received on average 306 MBq (range 233–362 MBq) of [¹⁸F]MK-9470 in slow intravenous injection under standardized injection circumstances (supine, low ambient noise, dimly lit room). Starting 90 min after injection, a dynamic PET emission scan was started in 3D mode (HR+, Siemens, Germany), consisting of six frames with frame duration of 300 s. Total scan duration was 30 min. The subject's head was placed in a head holder and fixed using a vacuum mask to avoid excessive head movement. Images were reconstructed using a 3DRP algorithm including scatter and measured attenuation correction (⁶⁸Ge source).

Image processing and data analysis

Correction for motion between frames was performed using the realignment module in SPM2 (Wellcome Department of Cognitive Neuroscience, London, UK). Modified standardized uptake value images of [¹⁸F]MK-9470 binding were calculated using PMOD version 2.7 (PMOD Inc., Zurich, Switzerland) where the activity concentration at each voxel was divided by the amount of tracer injected and normalized to the subject's weight (kg): modified standardized uptake

Table 1 Patient characteristics

Number	Age	Sex	Initial precipitating injury	Age at onset (years)	Number of seizures in month before scanning	Latency since last seizure before scanning (days)	MRI	Interictal EEG	Ictal EEG	SISCOM	FDG PET	Relative CB1R in temporal lobe cluster	Outcome surgery	Pathology	Antiepileptic drugs
1	28	F	Febrile seizures	6	2	26	HS R	Temporal R	Temporal R	Temporal R	Temporal R	0.92	Engel I	HS	Carbamazepine
2	70	M	Limbic encephalitis	55	0	67	HS R	Temporal R			Temporal R; parietal L	0.93			Carbamazepine
3	44	F	Concussion	15	0	144	HS L	Normal				0.96			Carbamazepine, lamotrigine
4	60	F	Febrile seizures	23	0	87	HS L	Temporal L	Temporal L	Temporal L	Bitemporal	0.99	Engel II	HS	Levetiracetam, phenobarbital, phenytoin
5	59	M		50	5	0.75	HS R	Temporal R	Temporal R	Temporal R	Temporal R; parietal R	1.16	Engel II	HS	Levetiracetam, topiramate, phenobarbital
6	53	F		37	4	0.2	HS L	Temporal L			Temporal L	0.99	Engel I	HS	Carbamazepine, levetiracetam
7	39	F	Concussion	15	2	15	HS R	Temporal R	Temporal R	Temporal R	Temporal R	0.92	Engel I	HS	Topiramate, levetiracetam
8	57	M		2	1	32	HS L	Temporal R	Temporal R	Temporal R	Bitemporal, biparietal: L > R	0.97	Engel III	HS	Carbamazepine, phenytoin, tiagabine
9	33	M	Febrile seizures	10	7	0.2	HS L	Temporal L	Temporal L	Temporal L	Temporal L	0.99	Engel II	HS	Valproate, carbamazepine, clobazam, pregabalin, clonazepam
10	46	M		1	8	1.3	HS R	Temporal R	Temporal R	Temporal R	Temporal R	1.03			Carbamazepine, vigabatrin, levetiracetam
11	55	M		35	5	2	HS R	Temporal R	Temporal R	Temporal R	Temporal R	0.97	Engel II	HS	Levetiracetam, phenobarbital
12	28	M		13	4	0.3	HS L	Temporal L	Temporal L	Temporal L	Temporal L	1.03	Engel II	HS	Carbamazepine, levetiracetam

F = female; FDG = 2-[18F]-fluoro-2-deoxy-D-glucose; HS = hippocampal sclerosis; L = left; M = male; R = right.

value = {activity × [weight (kg) + 70 kg]/2}/injected dose (Sanabria-Bohorquez *et al.*, 2010). [¹⁸F]MK-9470 modified standardized uptake value images were co-registered to the subject's magnetic resonance image. The images of patients with hippocampal sclerosis on the right and left side were combined for analysis by left–right reversing images from patients with left-sided hippocampal sclerosis, in order to show the epileptogenic zone on the same (right) side in all patients. For this, PET and magnetic resonance images were co-registered and then flipped. Afterwards these images were spatially normalized to an in-house created CB1R template (Van Laere *et al.*, 2008) in standard Montreal Neurological Institute (MNI) space in SPM2 using non-linear warping (7 × 9 × 7 basis functions, 16 iterations). For further analyses, data were smoothed with a Gaussian kernel with a full-width at half-maximum of 10 mm.

Statistical parametric mapping analysis was performed comparing patients to controls in a categorical subject design with age and gender as nuisance variables, since CB1R availability is different for

males and females and shows an age dependency (Van Laere *et al.*, 2008). Proportional scaling was used and a relative analysis threshold of 80% of the mean was set to exclude non-grey matter activity. Data were explored using $P_{\text{height}} < 0.001$ (uncorrected). Clusters of significantly different CB1R binding were used as volumes of interest for further correlation analyses. Average modified standardized uptake values within the cluster volumes of interest were determined using PMOD and were normalized to the uptake in the whole brain.

Relative CB1R availability in the cluster volumes of interest was correlated with the latency since last seizure before scanning and the number of seizures in the month before scanning using a Spearman's correlation analysis (Statistica, version 9; Statsoft Inc., Tulsa, USA). Descriptive statistics and normality testing of the evaluated variables are shown in Table 2.

To evaluate the CB1R availability in individual patients, a Z-score map was created comparing the individual patient to controls. Z-score maps were explored at a threshold level of ≥ 2 SD compared to controls.

Table 2 Descriptive statistics and normality testing of the evaluated variables

	Median	IQR	Shapiro–Wilk's W test, P-value
CB1R availability in ipsilateral temporal lobe	0.98	0.07	0.02
Latency since last seizure (days)	8.50	48.98	0.002
Number of seizures in last month	3.00	4.50	0.27

IQR = interquartile range.

Results

There were no differences in global CB1R availability between patients with mesial temporal lobe epilepsy due to hippocampal sclerosis [modified standardized uptake value 1.07 ± 0.24 (mean \pm SD)] and controls (1.19 ± 0.20 ; $P = 0.13$).

In a voxel-based statistical parametric mapping group analysis, comparing patients with mesial temporal lobe epilepsy due to hippocampal sclerosis to controls, we found an increased CB1R availability in the ipsilateral temporal lobe extending from the inferotemporal to the mid- and superior temporal cortex (Fig. 1).

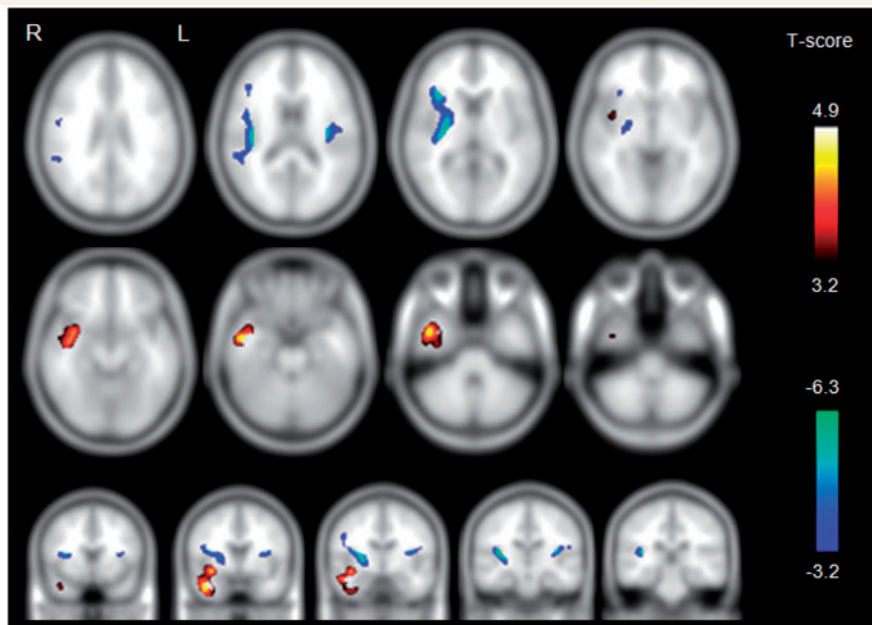


Figure 1 Statistical parametric mapping results showing average differences in the relative CB1R availability in patients with mesial temporal lobe epilepsy due to hippocampal sclerosis compared to healthy volunteers. Serial axial (*top two rows*) and coronal (*bottom row*) brain sections show an increase in CB1R availability in patients with mesial temporal lobe epilepsy due to hippocampal sclerosis in the ipsilateral temporal lobe extending from inferotemporal to mid-temporal and superior temporal cortex (yellow/red) and a decrease in the ipsilateral and contralateral superior insular cortex (green/blue). Significance at the voxel level is shown with a t -statistic colour scale. Results are projected on a spatially normalized T₁-weighted magnetic resonance imaging template. L = left; R = right.

Table 3 Cluster *P*-values and peak locations of the voxel-based group comparison of patients with mesial temporal lobe epilepsy due to hippocampal sclerosis and controls

	Cluster level		Voxel level		MNI peak coordinates and structure			
	$P_{\text{corrected}}$	k_E (mm ³)	T	$P_{\text{uncorrected}}$	x	y	z	Name
HS > HV	<0.001	10768	4.93	<0.001	44	4	−32	Ipsilateral inferotemporal cortex
HS < HV	<0.001	13 160	6.35	<0.001	36	−20	12	Ipsilateral insular cortex
	0.03	3208	5.27	<0.001	−34	−16	14	Contralateral insular cortex

HS = hippocampal sclerosis; HV = healthy volunteers; k_E = cluster extent.

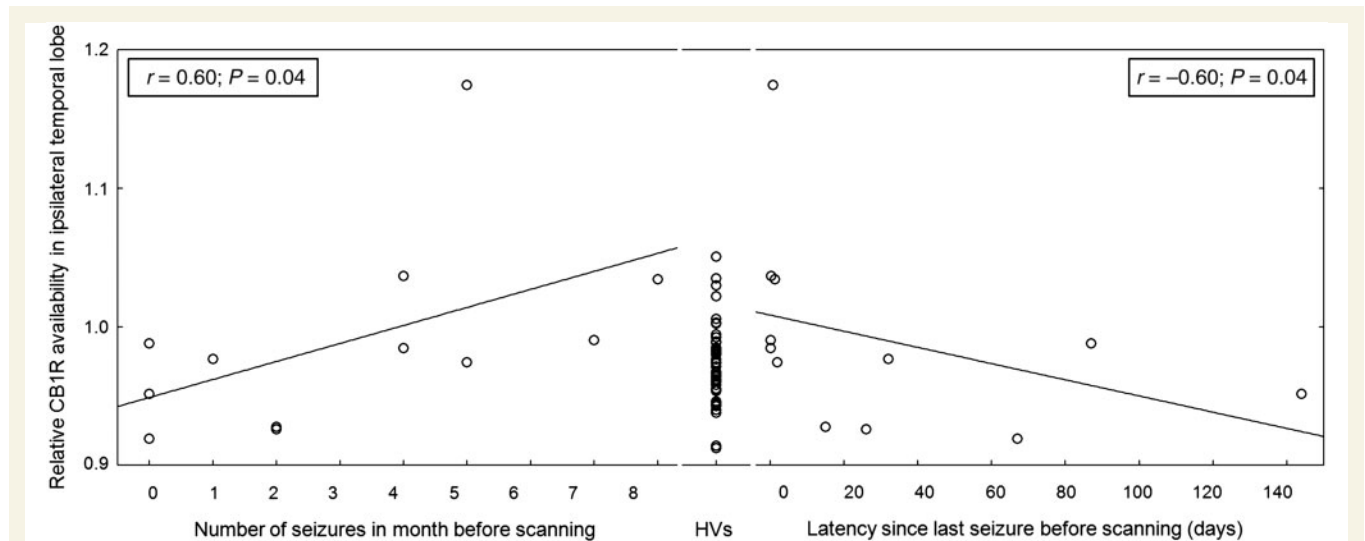


Figure 2 Scatter plot of the relative CB1R availability in the temporal lobe cluster in relation to the number of seizures in the month before scanning (*left*) and the latency since the last seizure before scanning in days (*right*). The relative CB1R availability is correlated positively to the number of seizures in the month before scanning ($r = 0.60$, $P = 0.04$) and negatively to the latency since the last seizure before scanning ($r = -0.60$, $P = 0.04$). As a reference, control values of the healthy volunteers (HVs) are plotted in the middle (0.97 ± 0.03 , mean \pm SD).

The CB1R availability was decreased in patients with mesial temporal lobe epilepsy due to hippocampal sclerosis in the ipsilateral and to a lesser extent the contralateral superior insular cortex (Fig. 1). A detailed description of the cluster characteristics and of the coordinates of the peak cluster locations is given in Table 3.

Evaluation of SISCOM images demonstrated that in the ipsilateral temporal lobe cluster, brain perfusion was increased during a seizure (average Z-score = 2.29, range 0.90–3.45). An increased ictal perfusion was also found in the ipsilateral superior insular lobe cluster, but this was less pronounced (average Z-score = 0.73, range 0.33–1.43). In the contralateral superior insular lobe cluster, ictal perfusion was decreased slightly on average, but variable between patients (average Z-score = -0.18 , range -1.03 to 0.85).

Correlation analysis using the cluster volumes of interest from the statistical parametric mapping analysis showed a positive correlation between relative CB1R availability in the ipsilateral temporal lobe and the number of seizures in the month before scanning ($P = 0.04$, $r = 0.60$) and a negative correlation with the latency since last seizure before scanning ($P = 0.04$, $r = -0.60$) (Fig. 2). There was no significant correlation between the relative

CB1R availability in the ipsi- or contralateral superior insular cortex and the latency since the last seizure before scanning or the number of seizures in the month before scanning.

Individual comparison of patients to controls using Z-score maps demonstrated a cluster of significantly increased CB1R availability (≥ 2 SD) in the ipsilateral temporal lobe in six of 12 patients. These six patients were scanned within 2 days of their last seizure (range 0.2–2 days). An example of such an individual Z-score map can be seen in Fig. 3. The cluster of individually increased CB1R availability in the ipsilateral temporal lobe coincides with the seizure onset zone, as presented by the relative ictal hyperperfusion in the patient's SISCOM image.

Discussion

In this article, we demonstrate that the endocannabinoid system in general and the CB1R more specifically show alterations in spatial relationship to the epileptogenic zone and in temporal relationship to seizures, suggesting a role in the pathophysiology of seizures. In patients with mesial temporal lobe epilepsy due to hippocampal sclerosis, we detected an increased CB1R availability in close

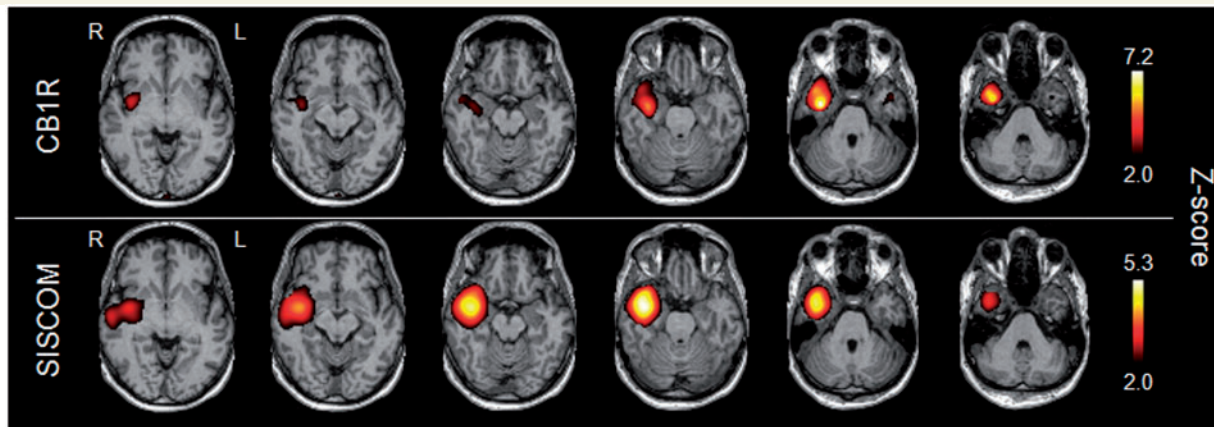


Figure 3 CB1R Z-score map (*top row*) and SISCOM (*bottom row*) of Patient 10 with right-sided hippocampal sclerosis. Serial axial brain sections show CB1R availability (yellow/red) in the right temporal lobe. This cluster coincides with the seizure onset zone, represented by the relative ictal hyperperfusion present in the patient's SISCOM image (yellow/red). Significance at the voxel level is shown with a Z-statistic colour scale. Results are projected on the patient's spatially normalized T₁-weighted magnetic resonance image. L = left; R = right.

relationship to seizures. This increase was present at the seizure onset zone, being the ipsilateral temporal lobe, in which an ictal hyperperfusion was demonstrated on the SISCOM images. The increase was also present on an individual basis when the patient was scanned within 2 days of a seizure. Likewise, the relative CB1R availability in the ipsilateral temporal lobe was positively correlated with the number of seizures in the month before scanning and negatively with the latency since last seizure before scanning. Since we used a cross-sectional study design in which each patient was scanned only once, it is possible that (i) seizures lead to an acute, transient increase in CB1R availability; (ii) a chronic, stable increase in CB1R availability predisposes to frequent seizures; and (iii) a combination of these two possibilities occurs together. To further elucidate our findings, a longitudinal study with repeated PET scans in the same patient is required. Using a longitudinal [¹¹C]flumazenil PET design, Bouvard and co-workers (2005) have demonstrated that there is a seizure-related short-term plasticity of benzodiazepine receptors in patients with mesial temporal lobe epilepsy, with lower B' (max) values in the epileptogenic hippocampus related to a shorter interictal period (Bouvard *et al.*, 2005). Using a longitudinal [¹¹C]diprenorphine PET design, Hammers *et al.* (2007) demonstrated that in patients with temporal lobe seizures, there is an increase of opioid receptor availability in the temporal pole and fusiform gyrus ipsilateral to the seizure focus following seizures. Within this region, there was a negative correlation between opioid receptor availability and log₁₀ time since last seizure before scanning, compatible with an early increase and gradual return to baseline (Hammers *et al.*, 2007).

We were unable to detect differences in the CB1R availability in the sclerotic hippocampus itself. In patients with mesial temporal lobe epilepsy due to hippocampal sclerosis, volume loss of the hippocampus will result in partial volume effects on PET imaging. Since we did not use partial volume correction in our study, this could account, at least in part, for this negative finding. However, we found a significant increase in CB1R availability in the

ipsilateral temporal lobe of patients with mesial temporal lobe epilepsy due to hippocampal sclerosis, who often have some degree of neocortical atrophy in addition to the hippocampal atrophy (Bernhardt *et al.*, 2010). Absence of partial volume correction in our study probably underestimated the detected increase in CB1R availability. *In vitro*, Ludanyi *et al.* (2008) have demonstrated a decreased level of CB1R messenger RNA and of CB1R density in the resected sclerotic hippocampus of patients who underwent epilepsy surgery.

Methodologically, quantification of CB1R availability was done using the brain radioactivity uptake parameter standardized uptake value, which does not incorporate the plasma input curve. CB1R standardized uptake values correlate to fractional uptake rate values, which represent CB1R density (Sanabria-Bohorquez *et al.*, 2010). In order to reduce the influence of a subject's weight on quantitative measurements (Thie *et al.*, 2007), we used modified standardized uptake values in the evaluation of the endocannabinoid system in patients that show the best correlation with fractional uptake rate values. Since only regional differences were found between epilepsy and control groups, it is unlikely that this would be a confound.

In the hippocampus, CB1Rs are present on both excitatory glutamatergic neurons and inhibitory GABA-ergic interneurons (Hoffman and Lupica, 2000; Hoffman *et al.*, 2010). Although PET imaging allows for *in vivo* imaging of CB1R in humans with epilepsy, it is unable to allocate the subpopulation of neurons on which this increased number of CB1Rs is present. The detected increased CB1R availability in epilepsy can, therefore, be interpreted in several ways. It may represent an acute on-demand activation of CB1Rs on glutamatergic neurons as an anti-epileptic and neuroprotective mechanism, or a long-term upregulation of CB1Rs on GABA-ergic neurons, which renders the epileptogenic zone hyperexcitable and vulnerable to epileptic seizures, or a combination of both.

In the first hypothesis, the increased number of CB1Rs would mainly be present on glutamatergic nerve endings, which can be

seen as a mechanism, induced by seizures, in order to protect neurons against hyperexcitability, further seizure activity and excitotoxicity (Marsicano *et al.*, 2003). Cannabinoid action would thus result in increased depolarization-induced suppression of excitation. It has been shown that in the hippocampus, depolarization-induced suppression of inhibition is the prominent cannabinoid-induced phenomenon in basic conditions and plays an important role in normal brain functions. When high-frequency action potentials occur, as is the case during a seizure, depolarization-induced suppression of inhibition is abolished completely (Foldy *et al.*, 2006) and a switch takes place towards depolarization-induced suppression of excitation (Ohno-Shosaku *et al.*, 2002). In several animal models, it has been demonstrated that an increase of the endocannabinoid tone by administration of endogenous as well as exogenous cannabinoid agonists has anti-convulsant properties (Wallace *et al.*, 2001, 2002; Blair *et al.*, 2006; Deshpande *et al.*, 2007; Naderi *et al.*, 2008).

In the second hypothesis, the increase represents a long-term upregulation of CB1R on GABA-ergic neurons, which renders the epileptogenic zone hyperexcitable and vulnerable to epileptic seizures. The increased cannabinoid transmission would then result in an increased suppression of inhibition, leading to hyperexcitability. An initial precipitating insult, like complex febrile seizures, might trigger pro-epileptogenic changes in the endocannabinoid system, which after a latent period, result in the development of spontaneous seizures. Chen and colleagues have shown that early administration of CB1R-antagonists during prolonged febrile convulsions may have an anti-epileptogenic effect (Chen *et al.*, 2007). After each seizure, these changes can reoccur and cause worsening of the seizures over time, as is the case in patients with mesial temporal lobe epilepsy due to hippocampal sclerosis (French *et al.*, 1993). More generally, the CB1R upregulation in the epileptogenic zone may then be a hallmark of acquired epilepsies characterized by an initial precipitating injury, of which mesial temporal lobe epilepsy due to hippocampal sclerosis is an example. Further studies of the CB1R availability therefore seem warranted in, for example, patients with acquired epilepsy after a traumatic brain injury.

Despite multiple drug treatment, a group of patients will continue to have frequent seizures. These patients can benefit from surgical resection of the seizure onset zone. In order to perform surgery, this seizure onset zone needs to be identified clearly. Despite the multitude of tests available, reliable detection of the seizure onset zone remains difficult in some patients. CB1R-PET performed within 1 or 2 days after a seizure may be an excellent imaging modality to visualize the seizure onset zone. Further studies are warranted.

In addition to an increased CB1R availability in the ipsilateral temporal lobe, we found a decreased receptor availability in the ipsilateral and contralateral superior insular cortex. Within the ipsilateral insular cortex, we detected an ictal hyperperfusion on the SISCOM images, which was, however, less pronounced than the ictal hyperperfusion in the seizure onset zone itself. In the contralateral insular cortex, the ictal perfusion was slightly decreased on average, but with significant variation between subjects. The superior insular cortex, therefore, is at the border of the transition zone between temporal lobe hyperperfusion-frontal lobe

hypoperfusion during complex partial seizures (Van Paesschen *et al.*, 2003). Dense connections exist between temporo-limbic structures and the insular cortex and seizures originating in the temporal lobe often invade the insular cortex (Isnard *et al.*, 2000). In the insular cortex, CB1Rs are expressed in moderate levels (Van Laere *et al.*, 2008) and are mainly present on GABA-ergic nerve terminals (Hill *et al.*, 2007). A decreased level of CB1Rs on these nerve terminals may thus result in an increased level of inhibition, which might contribute to prevention of seizure propagation. Using interictal 2-[¹⁸F]-fluoro-2-deoxy-D-glucose PET imaging and SISCOM during complex partial seizures in patients with mesial temporal lobe epilepsy due to hippocampal sclerosis, we were able to demonstrate that the ipsilateral frontal lobe is characterized by interictal hypometabolism and ictal hypoperfusion. We postulated that there is surround inhibition in the ipsilateral frontal lobe, which is a dynamic defence mechanism against propagation of complex partial seizures in mesial temporal lobe epilepsy due to hippocampal sclerosis (Nelissen *et al.*, 2006). From our data, we speculate that the endocannabinoid system in the insular region plays an important role in this inhibition of seizure propagation in mesial temporal lobe epilepsy due to hippocampal sclerosis. In other areas with frequent seizure propagation, no average group differences in CB1R availability were detected.

Conclusion

In summary, using *in vivo* [¹⁸F]MK-9470 PET imaging, we found a seizure-associated increase in CB1R availability at the seizure onset zone and a decrease in CB1R availability in the insular cortex in patients with mesial temporal lobe epilepsy due to hippocampal sclerosis, suggesting that changes in the endocannabinoid system modify excitability in different ways in the epileptic network in mesial temporal lobe epilepsy due to hippocampal sclerosis. Longitudinal studies and studies in other epilepsy syndromes are important to further elucidate the role of the endocannabinoid system in epilepsy.

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