

### Summary

The current aim of positron emission tomography (PET) investigation in pharmacoresistant epileptic patients is the *in vivo* study of the neurotransmission abnormalities underlying neuronal hyperexcitability, with the hope of better delineating the epileptogenic zone non-invasively. This approach is based on the binding of various radioligands on specific receptors, such as GABA<sub>A</sub>, serotonin, opiate receptors, or on the brain uptake of radioactive neurotransmitter precursors. Although promising studies have been reported, at the present state, the PET studies using these new tracers are confined to research in a few centres and their clinical role and utility in presurgical assessment of pharmacoresistant focal epilepsies are difficult to evaluate, partly due to the lack of large multicentric controlled studies. The new and very latest PET tracers studies will be reviewed.

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**Key words:** Epilepsy, PET, radiotracer, neurotransmission

### Neue PET-Tracer bei Epilepsie

Das Ziel der Positron-Emissions-Tomographie (PET)-Studien in der Untersuchung von pharmakoresistenten epileptischen Patienten ist die *in vivo*-Charakterisierung von Neurotransmitteranomalien, die der neuronalen Übererregbarkeit zugrunde liegen, in der Hoffnung, so die epileptogene Zone besser zu bestimmen. Diese Methode basiert auf der Bindung von Radioliganden mit spezifischen Rezeptoren, wie GABA<sub>A</sub>, Serotonin, Opiat-Rezeptoren, oder auf der zerebralen Aufnahme von Neurotransmitter-Vorstufen. Obwohl viel versprechende Studienresultate berichtet wurden, sind PET-Untersuchungen mit neuen Tracern wenigen Forschungszentren vorbehalten, und ihre klinische Rolle und Nützlichkeit ist aufgrund mangelnder multizentrischer kontrollierter Studien noch schwierig zu beurteilen. Die neuesten PET-Tracer-Studien werden hier besprochen.

**Schlüsselwörter:** Epilepsie, PET, Radiotracer, Neurotransmission

### Les nouveaux traceurs PET en épileptologie

Un des buts actuels des investigations par tomographie à émission de positons (PET) chez les patients épileptiques pharmacorésistants est l'étude *in vivo* des anomalies de neurotransmission qui sous-tendent l'hyperexcitabilité neuronale, avec l'espoir de mieux

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délimiter, d'une manière non invasive, la zone épileptogène. Cette approche est basée sur la liaison de divers radioligands sur des récepteurs spécifiques, tels que les récepteurs GABA<sub>A</sub>, sérotonine, opiacés, ou sur la captation cérébrale de précurseurs de neurotransmetteur radioactifs. Bien que des études prometteuses aient été rapportées, à l'heure actuelle les études PET utilisant ces nouveaux traceurs sont limitées à de la recherche dans quelques centres et il est difficile d'évaluer leur rôle et utilité cliniques dans l'évaluation préchirurgicale des épilepsies focales pharmacorésistantes, en particulier en raison du manque de larges études multicentriques contrôlées. Cet article passe en revue les nouveaux traceurs PET existant pour les divers systèmes de neurotransmission.

**Mots clés :** Epilepsie, PET, radiotraceurs, neurotransmission

### Introduction

Positron emission tomography (PET) has been the first functional neuroimaging technique applied to presurgical evaluation of pharmacoresistant focal epilepsies, in the late seventies, before MRI was available. It used the fluoro-deoxyglucose labeled with <sup>18</sup>F isotope (<sup>18</sup>FDG) to obtain images of interictal brain glucose metabolism. It was particularly useful in patients with a normal brain CT scan, showing a focal interictal glucose hypometabolism. Today <sup>18</sup>FDG PET remains a routinely used examination in the presurgical assessment of drug refractory focal epilepsies. Another objective of PET investigation which has been progressively developed, is to get images of neurotransmission abnormalities underlying neuronal hyperexcitability. This requires radioactively labeled tracers which are either ligands of specific receptors or neurotransmitter precursors. Thus, one of the most promising applications of PET in epilepsy studies consists of imaging the distribution of brain receptors in the interictal state. The most widely used PET ligand in epilepsy at present is the selective antagonist of GABA<sub>A</sub> receptors, [<sup>11</sup>C]-flumazenil. A localized reduction of [<sup>11</sup>C]-flumazenil binding, closely correlating with the side and site of seizure onset, is usually observed in patients with refractory focal seizures. This reduced binding is thought to largely reflect an underlying neuronal loss, as demonstrated in temporal lobe epilepsy associated with mesial temporal sclerosis (review in [1]).

Less known specific ligands or precursors usable for PET studies are now available for various neurotransmitter and neuromodulator systems including the serotonin, dopamine, glutamate/NMDA, nicotinic acetylcholine, adenosine and opioid systems. The PET studies using these new tracers are confined to research in a few centers and are

not in routine clinical use. Obviously, no epilepsy center has the capacity of developing all the tracers. Large multicentric controlled studies are lacking and to date no PET technique has proved its capacity to map the epileptogenic zone with enough precision to guide cortical resection. This paper will present an overview of the new tracers and describe their potential in clinical and experimental epileptology. Most of the reported studies have used statistical parametric mapping (SPM) for the statistical analyses of the data.

## I. Serotonin system

The serotonin system originates from the raphe nuclei, with widespread projections to the whole CNS. Studies in experimental models of epilepsy have suggested an inhibitory role of serotonin (5-HT) on epileptiform discharges [2, 3] and antiepileptic and anticonvulsant properties of 5-HT<sub>1A</sub> receptor activation in rodents [4]. The anti-seizure effects of 5-HT<sub>1A</sub> receptor activation is blocked by the highly selective 5-HT<sub>1A</sub> antagonist WAY-100635 [5]. It is interesting to note that enhanced serotonergic innervation has been described in the epileptogenic tissue of patients with cortical dysplasia [6].

### I.1. $\alpha$ [<sup>11</sup>C]methyl-L-tryptophan (AMT)

Serotonin is synthesized from the neutral amino acid L-tryptophan. Several lines of evidence support the validity of alpha-methyl tryptophan (AMT) as a tracer for measuring the rate of serotonin synthesis. PET studies using AMT have been performed in different epileptic populations. One of the main advantages of using PET with AMT for imaging in epilepsy is that it shows increased (rather than decreased) uptake in epileptic foci. The basis for increased AMT uptake in patients with epilepsy has not been completely elucidated.

Several studies have demonstrated the unique ability of AMT PET to successfully identify the epileptogenic tuber(s) in patients with tuberous sclerosis and intractable epilepsy [7 - 10]. AMT PET scanning shows locally increased uptake of AMT in and around the epileptogenic tuber, while it shows normal or decreased uptake in non-epileptogenic tubers [9]. These findings are not related to non-specific changes in perfusion or metabolism (lack of changes with interictal markers of blood flow or markers of metabolism). In a study of 17 children who underwent resective epilepsy surgery following AMT PET, the tuber with the highest uptake was located in an ictal EEG onset region in each patient [10]. Tubers with at least 10% increase of AMT uptake proved to be epileptogenic based on intracranial EEG and outcome criteria. The different studies demonstrated that resection of tubers with increased AMT uptake is essential to achieve seizure-free surgical outcome in these patients.

The occurrence of increased AMT uptake is higher in

patients with histologically proven cortical dysplasia compared to those with nonspecific pathological changes (i.e. gliosis) [11]. This correlates with previous human epileptic tissue studies showing serotonergic hyperinnervation in dysplastic tissues [6]. In patients with intractable epilepsy and cortical dysplasia, the increased uptake of AMT was shown to be highly co-localized to the area of neocortical seizure onset defined on electrocorticography. Remote cortex involved in seizure propagation does not appear to show increased uptake on AMT PET images. In contrast, the regions of reduced metabolism on <sup>18</sup>F-DG PET are widespread and nonspecific.

Increased AMT uptake was also found in a very high proportion of epileptogenic brain tumors, including low-grade gliomas and dysembryoplastic neuroepithelial tumors, but it is not always related to epileptogenicity as it has also been observed in some gliomas not associated with seizures [12].

It appears that AMT PET has a lower sensitivity for the localization and lateralization of epileptic foci in patients with cryptogenic focal epilepsy. However increased focal uptake of AMT may be observed in a proportion of patients with no detectable lesion on MRI and can be a valuable addition to current methods of investigation [13]. One study showed that AMT PET might be useful for lateralizing the epileptic focus in patients with temporal lobe epilepsy (TLE) and normal hippocampal volumes: an increased AMT uptake was shown in the hippocampus ipsilateral to the seizure focus in a group of seven TLE patients with normal hippocampal volumes [14]. However other larger studies are needed to further substantiate the clinical use of AMT PET in evaluation of patients with suspected TLE and no signs of hippocampal sclerosis on MRI.

Lastly, AMT PET was shown to be a useful imaging approach for identification of non-resected epileptic cortex in patients with a previously failed neocortical epilepsy surgery [15]. It is proposed to wait at least 2 months after surgery before scanning the patients.

The conclusion of these studies is that AMT is a useful tracer in the presurgical evaluation of patients with epilepsy and that it displays a particularly high specificity for the dysplastic lesions of tuberous sclerosis or cortical dysplasia.

### I.2. Ligands of 5-HT<sub>1A</sub> receptors

The 5-HT<sub>1A</sub> receptors constitute the best characterized subtype of currently known 5-HT receptors.

#### I.2.1. [<sup>11</sup>C]-WAY-100635 and [<sup>18</sup>F]-FCWAY

[<sup>11</sup>C]-WAY is an antagonist ligand of 5-HT<sub>1A</sub> receptors. It is very specific with a much higher affinity than endogenous serotonin for 5-HT<sub>1A</sub> receptors (K<sub>d</sub> in the range of 20 pmol), so [<sup>11</sup>C]-WAY does not interact with serotonin.

PET using [<sup>11</sup>C]-WAY-100635 was performed in patients

with severe mesial temporal lobe epilepsy (MTLE) to test the hypothesis that in MTLE there is involvement of serotonin systems outside of mesial structures, suggesting a mechanism for affective symptoms in this population [16]. Fourteen patients (6 with left-, 8 with right-sided mesial temporal lobe focus) and 14 controls were studied. The 5-HT<sub>1A</sub> receptor binding potential was calculated for hippocampus, amygdala, orbitofrontal, insular, lateral temporal, anterior cingulate cortex, raphe nuclei, and in two regions presumably uninvolved in the epileptogenic process (parietal, and dorsolateral frontal neocortex). The 5-HT<sub>1A</sub> binding was significantly reduced in the epileptogenic hippocampus and amygdala ( $p = 0.0001$ ) in all patients, including the six with normal [<sup>18</sup>F]-FDG PET and MRI. It was also reduced in the anterior cingulate, insular, and lateral temporal cortex ipsilaterally to the focus, in contralateral hippocampus, and in the raphe nuclei. Thus the reduced 5-HT<sub>1A</sub> receptor binding potential was observed in the EEG focus and its limbic connections. According to the authors, the affective symptoms in MTLE may result from reductions in 5-HT<sub>1A</sub> binding in the insular and cingulate cortex. In conclusion, [<sup>11</sup>C]-WAY-100635 PET may provide additional information to EEG, [<sup>18</sup>F]-FDG PET, and MRI when evaluating patients with intractable seizures.

PET using [<sup>11</sup>C]-WAY-100635 was also performed in 12 patients with juvenile myoclonic epilepsy (Savic, personal communication, "The new and very new PET tracers in epilepsy", Satellite symposium to the 26<sup>th</sup> IEC, Orsay, Paris). There was a 25% reduction of 5-HT<sub>1A</sub> binding in the dorsolateral prefrontal cortex. It is worth noting that the same patients were investigated with MR spectroscopy showing reduction in N-acetylaspartate (NAA) in the dorsolateral prefrontal cortex, and also had impaired working memory. In addition, an unexpected finding was a bilateral reduction of [<sup>11</sup>C]-WAY-100635 binding in the hippocampus, despite hippocampal volumes which were not decreased and despite normal grey matter fraction. The authors suggest that 5-HT<sub>1A</sub> receptor binding could be a useful approach in future to detect potential hippocampal changes which are not visible on MRI in neocortical epilepsies, generalized epilepsies or in psychiatric disorders.

[<sup>18</sup>F]-FCWAY presents an affinity for 5-HT<sub>1A</sub> receptors comparable to that of the original WAY-100635 labeled with <sup>11</sup>C. A PET study with [<sup>18</sup>F]-FCWAY showed decreased temporal 5-HT<sub>1A</sub> binding ipsilateral to seizure foci in patients with TLE [17]. A complementary study demonstrated that decreased 5-HT<sub>1A</sub> binding in insula and mesial temporal structures ipsilateral to temporal lobe epileptic foci is not an artifact related to partial volume effect because of the mesial temporal sclerosis and structural atrophy [18]. The studies suggest that the receptor loss may be part of the initial phase of neuronal dysfunction in TLE, followed by hypometabolism and eventual structural atrophy. The decrease in 5-HT<sub>1A</sub> binding exceeded both <sup>18</sup>FDG hypometabolism and hippocampal atrophy, and could be detected in mesial temporal regions in patients with normal MRI. Thus [<sup>18</sup>F]-FCWAY PET might be particularly useful for early detection of functional abnormalities in TLE patients.

## I.2.2. [<sup>18</sup>F]-MPPF

MPPF (4-(2'-methoxyphenyl)-1-[2'-[N-(2"-pyridinyl)-p-fluorobenzamido]ethyl]-piperazine) is another selective antagonist of 5-HT<sub>1A</sub> receptors. It has an affinity close to that of endogenous serotonin for 5-HT<sub>1A</sub> receptors and is thus sensitive to endogenous serotonin variations. Thus a decrease of [<sup>18</sup>F]-MPPF binding can be interpreted as reflecting either a decrease in receptor density or an increase of endogenous serotonin, resulting in a competition for receptor binding by the radioligand.

PET studies with [<sup>18</sup>F]-MPPF carried out in a group of TLE patients with hippocampal ictal onset showed significant decreases ipsilateral to the epileptogenic zone in the hippocampus, temporal pole, insula and temporal neocortex [19, 20]. It was concluded from these data that the decrease in 5-HT<sub>1A</sub> receptor binding in epileptic patients could reflect the loss of neurons in the hippocampus. However, this interpretation has recently been challenged by the report in epileptic foci of an increase in P-glycoprotein, an ATP-driven transmembrane efflux pump, known to strongly regulate the penetration of [<sup>18</sup>F]-MPPF in the brain [21]. The binding of [<sup>18</sup>F]-MPPF might be modified by extracellular 5-HT levels, internalization of 5-HT<sub>1A</sub> receptors and the expression of P-glycoproteins. So, although [<sup>18</sup>F]-MPPF proves a successful 5-HT<sub>1A</sub> receptor imaging agent, the development of novel 5-HT<sub>1A</sub> PET radioligands will be required to further characterize 5-HT neurotransmission and 5-HT<sub>1A</sub> receptors in human brain [22].

## II. Dopamine system

Studies in animal models and epileptic patients have suggested that circuits of the basal ganglia may control epileptic seizures and that striatal dopaminergic transmission plays a key role in seizure interruption [23]. In addition, there is evidence from clinical experience that antagonizing D2 receptors lowers seizure threshold.

### II.1. [<sup>18</sup>F]-fluoro-L-Dopa

[<sup>18</sup>F]-fluoro-L-Dopa is a radiotracer that permits measurements of presynaptic dopaminergic function. A [<sup>18</sup>F]-fluoro-L-Dopa PET study was performed in patients with a ring chromosome 20 epilepsy, characterized by long-lasting seizures suggesting a dysfunction in the seizure control system. It showed a significantly decreased uptake in both putamen and caudate nucleus, indicating that a dysfunction of the striatal dopamine neurotransmission may impair the mechanisms that interrupt seizures [24].

Thereafter, patients with generalized seizures and patients with focal seizures related to hippocampal sclerosis were studied [25]. There was a decreased [<sup>18</sup>F]-fluoro-L-DOPA uptake, especially in the substantia nigra bilaterally, in all patients. [<sup>18</sup>F]-fluoro-L-DOPA uptake was also decreased in the putamen, bilaterally, in patients with gene-

ralized seizures and unilaterally, ipsilateral to the hippocampal sclerosis, in patients with focal seizures. This study provides further evidence that the basal ganglia, and especially the substantia nigra, are involved in human epilepsy.

## II.2. [<sup>18</sup>F]-Fallypride

PET using the highly selective, high-affinity, dopamine D2/D3-receptor ligand [<sup>18</sup>F]-Fallypride is suitable for measuring D2/D3 receptor availability in the extrastriatal regions of the brain. A group of seven patients with TLE and hippocampal sclerosis, was compared with a group of age-matched controls [26]. Compared with controls, [<sup>18</sup>F]-Fallypride binding potential was significantly decreased in the epileptogenic temporal lobe in all patients. On the analysis of regions of interest, this reduction was evident in areas surrounding the seizure onset zone, at the temporal pole (-34%) and the anterior part of the lateral temporal lobe (-33%). Although the hippocampal [<sup>18</sup>F]-FDG uptake (-8%) and hippocampal MR volume (-35%) were significantly reduced, no significant decrease of [<sup>18</sup>F]-Fallypride binding potential was found in the hippocampal area. Thus, D2/D3-receptor binding is reduced at the pole and in lateral parts of the epileptogenic temporal lobe in patients with mesial TLE and HS. This area of decreased binding might correspond to “the irritative zone”, suggesting that D2/D3 receptors might play a specific role in the pathophysiology of mesial TLE.

## III. Glutamate / NMDA system

Glutamate is the principal excitatory neurotransmitter in the human brain. Its receptors are divided into ionotropic and metabotropic receptors. N-methyl-D-aspartate (NMDA) receptors form a subclass of ionotropic glutamate receptors. Enhanced excitatory transmission plays a central role in the generation of seizures and the development of epilepsy. The NMDA receptors have been studied in human epileptogenic brain with conflicting results. Increased as well as decreased receptor binding has been reported in epileptogenic tissue.

### III.1. [<sup>11</sup>C]-CNS 5161

CNS 5161 is an NMDA antagonist that binds to NMDA ion channel sites with high affinity. [<sup>11</sup>C]-CNS 5161 is currently developed as a potential PET tracer. Four healthy control subjects and a single pilot case with mesial TLE were scanned with this tracer (Hammers, “The new and very new PET tracers in epilepsy”, Satellite symposium to the 26<sup>th</sup> IEC, Orsay, Paris). While hippocampal volume on the affected side was reduced by 27% compared to the contralateral side, [<sup>11</sup>C]-CNS 5161 volume of distribution was reduced by only 13%. This may indicate an actual in-

crease in open NMDA channels per volume unit of tissue on the epileptogenic side. Larger studies, with partial volume correction, are needed.

Another study aimed to correlate hippocampal [<sup>11</sup>C]-CNS 5161 volume of distribution and memory performances in eight healthy volunteers, with the hypothesis that the number of “active” NMDA receptors would be positively correlated with memory performances [27]. The predicted association between hippocampal [<sup>11</sup>C]-CNS 5161 volume of distribution in the “resting” state and (verbal) memory performance was found, so NMDA activity might be a general marker for the ability to learn new material. Thus initial evaluations have yielded promising results, with a potential usefulness for both group studies and longitudinal studies. However, the two performed pilot scans in patients with epilepsy have highlighted the difficulties in modeling, due to the binding in vivo being neither clearly reversible nor irreversible, and due to the current restricted knowledge of in vivo binding behaviour.

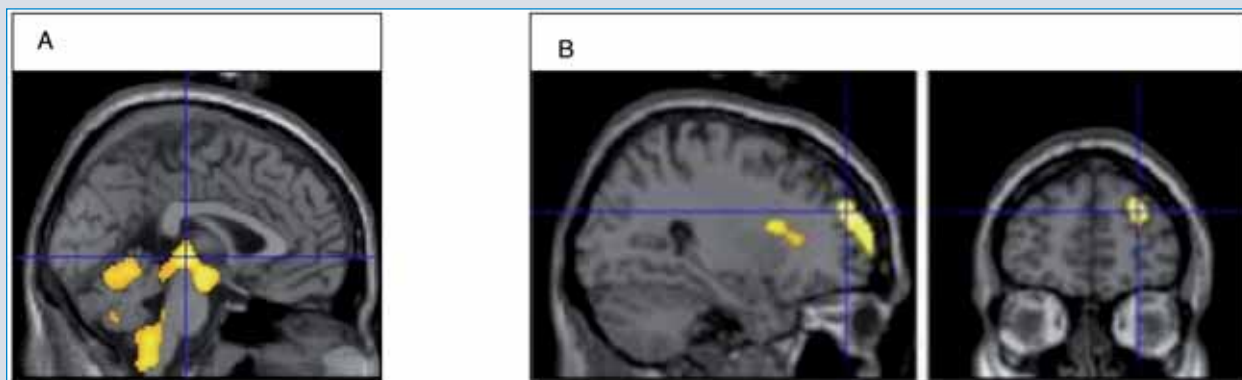
### III.2. [<sup>11</sup>C]-ketamine

Ketamine is an anaesthetic which binds specifically and reversibly to the PCP-recognition site of the NMDA receptor in a non-competitive manner. Receptor affinity (K<sub>d</sub>) is in the μmol range. PET studies, especially in monkeys, but also in humans, have shown that the distribution of [<sup>11</sup>C]-S-ketamine corresponds to regions with high density of glutamate receptors.

Eight patients with mesial TLE were evaluated by PET using [<sup>11</sup>C]-ketamine [28]. The uptake of [<sup>11</sup>C]-ketamine in the temporal lobe of ictal onset was compared with the contralateral side and correlated to changes in regional glucose metabolism measured by PET with FDG. A side-to-side comparison revealed a 9-34% reduction of tracer radioactivity in the temporal lobes of ictal onset. The magnitude and distribution of the reduction were similar to the metabolic pattern seen on PET scans with FDG. This reduction may reflect reduced NMDA receptor density, reduced perfusion, focal atrophy, or other factors. Further studies with correction for partial volume effects and perfusion differences are needed.

## IV. Nicotinic cholinergic system

About ten years ago, mutations were identified in one form of familial non lesional focal epilepsy, the autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), in genes coding for two different subunits of the neuronal nicotinic acetylcholine receptor (nAChR), respectively the α4 and the β2 subunits. To date, such mutations have been found in nearly fifteen families [29]. These subunits are known to assemble and form the main brain nicotinic receptor subtype in humans. The nAChRs are excitatory receptor channels permeable with cations (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>), widely distributed throughout the brain. Most of these



**Figure 1.** A) SPM analysis of [18F]-F-A-85380 PET hyperfixation in ADNFLE patients, corresponding to regions of increased density of nicotinic receptors (patients  $n = 8$ ; controls  $n = 7$ ;  $P$  uncorrected  $< 0.001$ ,  $P$  corrected at cluster level  $< 0.05$ ). These images focus on the mesencephalic cluster, with the blue cross centered on the voxel with the highest Z score ( $Z = 4.63$ ; MNI coordinates:  $-2 -22 0$ ). This voxel is located in the epithalamus. The cluster extends in the ventral mesencephalon. B) SPM analysis of [18F]-F-A-85380 PET hypofixation in ADNFLE patients, corresponding to the regions of decreased density of nicotinic receptors (patients  $n = 8$ ; controls  $n = 7$ ;  $P$  uncorrected  $< 0.001$ ,  $P$  corrected at cluster level  $< 0.05$ ). The right side is on the right on the coronal MRI image. The hypofixation is located in the right dorsolateral prefrontal region.

receptors are presynaptic and have a neuromodulatory role consisting of an enhancement of the release of GABA, glutamate, dopamine, norepinephrine, serotonin or ACh.

PET study of nAChRs offers a unique opportunity to investigate some *in vivo* consequences of the molecular defect in ADNFLE patients. Formerly, the ligand used for the nAChRs was nicotine labeled with  $^{11}\text{C}$ . But its cerebral fixation was flow-dependent and partially nonspecific [30]. Later, the 2-Fluoro-A-85380 (3-[2(S)-2-azetidinyImethoxy]pyridine) labeled by a positron emitter isotope, the fluorine-18, was synthesized at Orsay CEA [31]. The F-A-85380 has remained a ligand with a high affinity and specificity for the central  $\alpha 4\beta 2$  nAChRs. The brain tracer concentration reflects the receptor concentration [32].

A PET using [ $^{18}\text{F}$ ]-F-A-85380 was performed in a group of 8 patients with ADNFLE carrying a mutation in a nAChR subunit, in comparison with a group of 7 age-matched healthy volunteers [33]. Patients and volunteers were all non-smokers. Parametric images of volumes of distribution were generated using the ratio between brain tissue concentration and the unchanged plasma concentration. The images showed a clear difference in the pattern of the nAChR density in the brains of the patients compared to the healthy volunteers. The volumes of distribution calculated on several brain regions delineated on individual MRI of patients with ADNFLE and of control subjects revealed a significant increase (between 12 and 21%,  $p < 0.05$ ) in the patients in the mesencephalon, the pons and the cerebellum. Statistical parametric mapping (SPM) confirmed clear regional differences between patients and controls: patients had increased nAChR density in the epithalamus, ventral mesencephalon and cerebellum, but decreased nAChR density in the right dorsolateral prefrontal region. In 5 patients who underwent an additional [ $^{18}\text{F}$ ]-FDG PET experiment, hypometabolism was observed in the neighbouring area of the right orbitofrontal cortex. The demon-

stration of a regional nAChR density decrease restricted in the prefrontal cortex, despite the known distribution of these receptors throughout the cerebral cortex, is consistent with focal epilepsy involving the frontal lobe. In addition, based on the known biochemical and cellular circuits in the brainstem, these results suggest that the nAChR density increase in the mesencephalon is involved in the pathophysiology of ADNFLE through the role of brainstem ascending cholinergic systems in arousal. An important step now, is to extend this PET examination to other forms of epilepsy, to confirm the specificity of the above-mentioned results for ADNFLE.

## V. Adenosine system (A1 adenosine receptor)

Adenosine is different from regular transmitters: it is not released in a vesicular way, not released in synapses. Instead, it is produced in the cell like the “sweat” of the cell. Whenever the cell has to work, adenosine production increases intra and extra-cellularly, activating the modulatory adenosine receptors. There are four different types of receptors, which have different affinities for adenosine. The receptors with the highest affinity are the A1 and A2A subtypes. The decision to choose the A1 adenosine receptors for tracer developments was mainly made because the A1 receptor is widely distributed throughout the brain.

In cases of high energy demand, such as in the early phases of an epileptic seizure, there is a massive increase of adenosine that is transported from the inside to the outside of the cell and thus can activate A1 receptors. It has been shown in animal models in the last two decades that the activation of A1 receptors increases activation of inhibitory G proteins and then helps stopping seizure activity. Adenosine is considered to be responsible for seizure arrest and for post-ictal refractoriness and thus

appears to be an endogenous antiepileptic regulator. The deficiencies within this system might result in a higher susceptibility for seizures or epileptogenesis. Most studies report reductions of A1 receptor density in experimental epilepsy models and in human post-mortem brain material of patients with epilepsy.

The radiotracer available for the A1 adenosine receptor is CFPFX, which stems from the same group as caffeine (caffeine being a non-selective blocker of adenosine receptors). CFPFX is fluorinated ( $^{18}\text{F}$ -CFPFX). It has relatively high affinity of 1.3 nM with rather high selectivity: A1/A2A >700. In the human brain, there is a high uptake within the striatum, the caudate nucleus, the putamen, part of the medial anterior thalamus and neocortical regions.

A study performed in a F98 rat model for brain tumors showed that there was an increased density of adenosine A1 receptors surrounding the tumor as well as surrounding the necrosis which is visible in the tumor [34]. The upregulation of A1 receptors is primarily on astrocytes.

A PET study using  $^{18}\text{F}$ -CFPFX in a patient with a glioma also revealed increases in A1 adenosine receptor density in the immediate vicinity of the tumor (invasion zone of the tumor), similar to the findings in the rat. However, in contrast to the rat findings, there was a decrease of A1 receptor binding surrounding this zone of increased receptors. This zone of "reduction of inhibitory capacity" could contribute to tumor-associated epilepsy. So the density of A1 receptors is within the normal range in the tumour, increased in the immediate peri-tumoral zone and decreased in the extra-tumoral area, which may result in an increased excitability of the brain.

Two patients with TLE have been studied (Bauer, "The new and very new PET tracers in epilepsy", Satellite symposium to the 26<sup>th</sup> IEC, Orsay, Paris). In the first case, including unilateral hippocampal sclerosis, there was a reduction of the hippocampal  $^{18}\text{F}$ -CFPFX signal on the sclerotic side. In a second case of TLE plus with dystrophic changes seen in the neocortex, lateralized decreased signal was observed compared to the contralateral side and compared to control levels. These data are not partial volume corrected.

It has to be noted that in autoradiographic studies of surgically resected hippocampi, densities were far lower than in control samples.

## VI. Opioid system

The opioid receptors can be classified into at least three types:  $\mu$ -,  $\delta$ - and  $\kappa$ -receptors. Opioid peptide release is calcium-dependent and requires high frequency neuronal firing; thus opioid peptides act as mediators of use-dependent synaptic activity and as co-transmitters to modulate the actions of the primary transmitter [35]. Opioid receptor availability reflects endogenous opioid concentrations. Animal and limited human data suggest an important anticonvulsant role for opioid peptides and

their receptors. Exogenously applied opioids have predominantly inhibitory actions on neuronal activity and transmitter release throughout the CNS. There is a large body of animal data showing that endogenous opioid release may occur following induced and spontaneous seizures and that increased opioid neurotransmission has an anticonvulsant role. However, the human relevance of these studies can only, at best, be inferential.

The tracer diprenorphine (DPN) is a non-selective partial agonist, which has similar affinity for  $\mu$ -,  $\delta$ - and  $\kappa$ -receptors. It is displaced by endogenous opioids [36]. It shows high binding to basal ganglia, amygdala, and layers V and VI of the cerebral cortex.

A recent study aimed to provide direct human in vivo evidence for changes in opioid receptor availability following spontaneous seizures [37]. Nine patients with refractory TLE were scanned by PET using  $^{11}\text{C}$ -DPN within hours of spontaneous temporal lobe seizures (median interval: 8.5 h postictally, range: 1.5 - 21.3 h). A second scan was acquired days to weeks later, after as long a seizure-free period was achievable in a given patient, and served as an intrasubject control (corresponding to interictal binding). A regionally specific increase of opioid receptor availability was evident following seizures in the temporal pole and fusiform gyrus ipsilateral to the seizure focus. Thus this study confirmed changes in opioid receptor availability in the hours following seizures, suggesting an important role of the opioid system in seizure control. On the contrary, previous studies performed during reading-induced seizures and absences demonstrated decreased  $^{11}\text{C}$ -DPN binding [38, 39]. Taking together the results of these previous studies and the recent study, the authors suggest that "synaptic opioid levels increase at the time of seizures, leading to a reduction in  $^{11}\text{C}$ -DPN binding, and that this is followed by a gradual recovery of available surface receptors with an overshoot over basal levels which is detected by PET about 8 h after seizures, with a gradual return to normal levels during the interictal phase".

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