Regional Reductions in Serotonin 1A Receptor Binding in Juvenile Myoclonic Epilepsy

Anna Meschaks, MD; Per Lindstrom, MD, PhD; Christer Halldin, PhD; Lars Farde, MD, PhD; Ivanka Savic, MD, PhD

**Background:** Juvenile myoclonic epilepsy (JME) is classified as primarily generalized epilepsy and as such is assumed to lack an anatomic substrate. Although neurochemical abnormalities are probable, few studies have investigated whether they exist in JME. Animal data and the high incidence of myoclonic seizures in serotonin-intoxicated patients suggest that the serotonin system may be disturbed in JME.

**Objective:** To test the hypothesis that JME is associated with a disturbed serotonin system and that this disturbance could be reflected in altered serotonin 1A receptor binding.

**Design:** The serotonin 1A receptor binding potential (BP) was measured with positron emission tomography and serotonin 1A receptor antagonist carbonyl–carbon 11–WAY-100635. The BP was calculated using a reference tissue model in several limbic and neocortical regions and the raphe nuclei.

**Setting:** Epilepsy clinics of the Karolinska University Hospital, Stockholm, Sweden.

**Patients:** Eleven patients with JME and 11 controls were studied.

**Main Outcome Measure:** Serotonin 1A receptor BP calculated in a set of volumes of interest.

**Results:** The patients with JME showed a reduced BP in the dorsolateral prefrontal cortex, raphe nuclei, and hippocampus.

**Conclusions:** The observed reductions in serotonin 1A receptor BP suggest that the serotonin system is affected in JME. Although the data give no definitive information about underlying mechanisms, they provide a strong argument for the view that not all brain regions are homogeneously involved in this condition, further questioning the current classification of primarily generalized epilepsy.

*Arch Neurol.* 2005;62:946-950

---

The term idiopathic generalized epilepsy describes a group of epilepsy syndromes with a nonfocal mechanism of onset and no identifiable cause other than a genetic predisposition. Juvenile myoclonic epilepsy (JME) represents a common form of idiopathic generalized epilepsy. The major features are bilateral myoclonic jerks of pubertal onset, occasional generalized tonic-clonic seizures, and absences. Because no anatomic substrate has been identified, a normal computed tomogram or magnetic resonance image (MRI) is a prerequisite for the diagnosis of JME. Increased frontal lobe gray matter fraction and benzodiazepine receptor density and reduced prefrontal glucose metabolism in JME raises questions about this concept. Furthermore, recent magnetic resonance spectroscopy data suggest that at least a certain population of patients with JME has frontal lobe reductions of N-acetyl aspartate and elevations of combined glutamine and glutamate (Glx) levels. Together, these findings strongly indicate identifiable frontal lobe abnormalities. Although they may explain a poor performance in frontal lobe tests and certain stereotyped personality traits in patients with JME, further investigations are needed to explain the pathophysiology of myoclonic jerks.

The epileptic myoclonus is thought to be primarily cortical. At a neurotransmission level, evidence exists that serotoninergic processes may be involved in the pathophysiology of myoclonus. For example, myoclonus is a frequent consequence of serotonin intoxication, and the serotonin 1A receptor antagonist methysergide is reported to reduce, and the serotonin 1A receptor agonist to induce, myoclonic jerks.

Central serotoninergic neurons originate from the raphe nuclei and innervate the hypothalamus, limbic system, striatum, and neocortex. The most extensively investigated of the 14 serotonin re-
The serotonin 1A receptor is the serotonin 1A receptor, which can be either presynaptic or postsynaptic. Presynaptic serotonin 1A autoreceptors are highly concentrated on cell bodies in the raphe nuclei. They mediate inhibition of neuronal firing and release of serotonin in all projection areas, thereby regulating serotonergic activity.

In human subjects, serotonin 1A receptor binding can be examined in vivo with positron emission tomography (PET) and the radioligand carbonyl–carbon 11–WAY-100635. We hypothesized that JME may be associated with a disturbance (a hyperreactivity) of serotonergic neurons, leading to altered serotonin 1A receptor binding. Changes were primarily expected in the dorsolateral prefrontal cortex, motor cortex, and raphe nuclei.

**METHODS**

**PATIENTS**

The study included 11 long-term consecutive patients (mean±SD age, 29±9 years; 6 women) treated from seizure onset at epilepsy clinics of the Karolinska University Hospital in Stockholm, Sweden. All of the patients were well known to the neurologists in charge (I.S., P.L.). The study was approved by the local human subject protection committee, and informed written consent was obtained from each patient.

The JME was diagnosed according to the International Classification of Epilepsies from 1989 based on seizure history, seizure semiology as described by relatives or recorded during hospitalization, and results of scalp electroencephalography, which all showed bilateral spike-and-wave or polyspike–and-wave activity. Patients had late childhood or teenage onset (mean±SD age, 14±2 years) of awakening myoclonic jerks. All had a history of generalized tonic and clonic seizures in addition to myoclonia. Four had experienced sporadic absences. No signs of focal onset were apparent, and all patients had normal results on computed tomography and routine MRI examination of the brain. None had a progressive condition, a history of status epilepticus, drug intoxication, or drug-related encephalopathy. One patient had newly diagnosed diabetes, which was controlled with a food regimen. The others were healthy apart from having seizures. All were treated with valproate sodium; one was also treated with lamotrigine.

The control group consisted of 11 right-handed, healthy volunteers (mean±SD age, 28±7 years; 6 women) without medication and with a normal MRI of the brain. They were not investigated with electroencephalography.

**MRI PROTOCOL**

All the patients were examined with a 1.5-T MRI system (Signa Advantage; General Electric Medical Systems, Milwaukee, Wis), using a previously described protocol.13,14 Possible hippocampal disease was evaluated from coronal T2 fast inversion recovery images (flip angle, 3°; 16 sections) and 3-dimensional spoiled gradient echo (2.5/0; 60 slices; 256×256 pixels; number of excitations, 1) images acquired perpendicular to the long axis of the hippocampus.

**MEASUREMENTS OF SEROTONIN 1A RECEPTOR BINDING**

All PET measurements were interictal, confirmed by online electroencephalography and the attending neurologist. The last seizure was at least 72 hours before the respective PET measurement. The PET system used was Siemens ECAT Exact HR (Siemens Medical Solutions, Knoxville, Tenn), with a spatial resolution of approximately 3.8 mm. Each patient received an intravenous bolus of approximately 6.75 mCi (250 MBq) of carbonyl–carbon 11–WAY-100635 (specific radioactivity, >1000 Ci/mmoll [37×106 MBq/mmol]). Blood samples were taken manually during the 63-minute scan. The time curve was corrected for radioactive metabolites and used for evaluation of possible differences in radioligand metabolism.

**ANALYSIS OF SEROTONIN 1A RECEPTOR BINDING POTENTIAL**

Serotonin 1A receptor binding potential (BP) was calculated in a set of volumes of interest (VOIs), which were first delineated on MRIs of a standard brain.16,17 They were then transferred to individual PET images, reformatted to perfectly fit with the standard brain (Figure 1). The VOI template included the hippocampus; amygdala; motor cortex; raphe nuclei; orbitofrontal, insular, and anterior cingulate cortex; lateral temporal cortex; dorsolateral prefrontal cortex; parietal cortex; and cerebellum (Figure 1). The motor cortex was delineated by means of the cytoarchitectonic area of the Human Brain Atlas program and the other VOIs as previously described.18,19

The BP of serotonin 1A receptors was calculated with the reference region version of the Logan graphical analysis. The cerebellum served as the reference region because it has a negligible density of serotonin 1A receptors.

**STATISTICAL ANALYSES**

Regional differences in serotonin 1A BP between patients and controls were tested with separate unpaired t tests (one for each VOI), using means of homologous regions as input values. Bonferroni correction was applied for the multiple comparisons of 10 different VOIs, yielding a P<.005.

In regions that showed changes, the BP was tested for a possible correlation with duration of seizures, seizure frequency, age, age at seizure onset, and the number of generalized tonic and clonic seizures during lifetime (Pearson simple regression, P<.05). Radioligand metabolite fractions were compared between cohorts (4 and 20 minutes after bolus injection) with unpaired t tests (P<.05).

**RESULTS**

**SEROTONIN 1A RECEPTOR BP**

The patients with JME showed significant reductions in serotonin 1A receptor BP in the dorsolateral prefrontal cortex (P=.002), raphe nuclei (P<.001), and hippocampus (P=.001) (Figure 2). No difference was found in other VOIs (Table). No correlations were found between regional BP and the clinical parameters.

To test whether the BP reduction in hippocampus could be attributed to atrophy, we delineated the hippocampus on individual coronal 3-dimensional MRIs, calculated the total brain volume with an in-house program, and compared the hippocampus–total brain volume ratios between patients and controls (unpaired t test, P<.05) in a post hoc analysis. No difference was observed (the mean±SD hippocampus volume was 2.3±0.4 cm3 in patients and 2.1±0.4 cm3 in controls).
In another post hoc analysis, based on the same MRIs, voxel-based morphometry was applied according to Good et al., using a limited search volume that covered the hippocampal regions (Talairach coordinates: \(x = -40\) to \(+40\); \(y = -42\) to \(+2\); \(z = -22\) to \(+3\)). After spatial normalization, 8-mm filtering, and segmentation into gray matter, white matter and cerebrospinal fluid controls and patients with JME were compared using SPM 99 statistical software (Wellcome Foundation, London, England). The T-threshold at pixel level was 0.001 (\(P < .05\)). The gray matter fraction of the hippocampus was significantly elevated in patients with JME, showing 2 peaks (Talairach coordinates: 16, −8, −12; z-level, 5.2; and −10, −8, −12; z-level, 4.4).

The mean±SD blood (plasma) concentration of carbonyl-11C-WAY-100635 did not differ between patients and controls (33%±15% for patients with JME and 39%±19% for controls at 4 minutes and 7%±5% for patients with JME and 8%±4% for controls at 20 minutes).

The present study examined whether the serotonin 1A receptor binding is altered in JME. The main finding was a significantly lower BP in patients compared with controls in the dorsolateral prefrontal cortex, raphe nuclei, and hippocampus. This finding supports recent notions that JME is a condition with behavioral manifestations that arise from cortical and subcortical circuits and adds a substantial argument for the view that juvenile myoclonic seizures have a located substrate.

As in many other PET studies of neuroreceptor binding, the data provide no definite conclusions about causality of the observed changes. Two alternative explanations, however, deserve a comment.

Serotonin 1A receptors in the hippocampus and frontal lobes could be down-regulated because of serotonin neuron hyperactivity and serotonin accumulation in these projection areas to raphe nuclei. Indeed, the epileptiform activity in JME is most pronounced over the frontocentral regions, and the degree of epileptiform activity has been found to correlate with reductions of serotonin 1A BP. Although not reported earlier, the currently observed hippocampal changes seem reliable considering the high sensitivity of carbonyl-11C-WAY-100635 PET hippocampal abnormalities. Neither hippocampal nor frontal lobe reductions in serotonin 1A BP seem, however, to reflect atrophy, because the re-
spective gray matter fractions appear elevated rather than reduced. Also antiepileptic medication seems an improbable explanation, considering that the changes were highly regional.

The finding in raphe nuclei deserves precaution, because this structure is small and its evaluation with PET, therefore, is hampered by partial volume effects. Such effects, however, increase the coefficient of variance in the measured values, which in the present context should mask a true group difference. The fact that our patients with JME showed reduced BP in raphe nuclei despite partial volume effects, therefore, provides an argument for a physiologic effect.

Although appealing, an interpretation of the present results as a mere effect of the serotonin neuron hyperactivity has several weaknesses. First, carbonyl-11C-WAY-100635 is a ligand with high affinity to the serotonin 1A receptor and not readily displaced by serotonin. It is, therefore, questionable whether a reduced BP reflects an elevation in serotonin concentration. Second, it is unlikely that the currently observed frontal lobe and hippocampal abnormalities are selective for the serotonin system. One possibility is that they reflect cortical dysplasia or other developmental disturbances. They could harbor changes of the serotonin system, leading to perturbations of autoreceptors in raphe nuclei.

These 2 explanations are difficult to separate on the basis of available information. At present, the evidence appears to strongly indicate that the serotonin system is affected in JME. The data confirm that frontal lobes are particularly affected in JME and point to the hippocampus as an additional target area. Together with previous data, these findings reject the notion that JME is a condition without anatomic substrates and emphasize the need for a reevaluation of the current classification of primary generalized epilepsy.

Accepted for Publication: October 19, 2004.

Correspondence: Ivanka Savic, MD, PhD, Division of Neurology, Karolinska University Hospital, Retziusväg 8, 171 77 Stockholm, Sweden (ivanka.savic-berglund@neuro.ki.se).

Author Contributions: Study concept and design: Savic. Acquisition of data: Meschaks, Lindstrom, Halldin, and Savic. Analysis and interpretation of data: Meschaks, Lindstrom, Farde, and Savic. Drafting of the manuscript: Meschaks and Savic. Critical revision of the manuscript for important intellectual content: Meschaks, Lindstrom, Halldin, Farde, and Savic. Obtained funding: Halldin and Farde. Administrative, technical, and material support: Halldin and Farde. Study supervision: Lindstrom and Savic.

Funding/Support: This study was supported by grant 98-14X-12599-01A from the Swedish Medical Research Council, Stockholm, Sweden, and by the Lundbeck Foundation, Lund, Sweden.

Acknowledgment: We acknowledge Anna-Lena Nordstrom, MD, PhD; Kjerstin Lind; Arsalan Amir; Jari Takiainen, PhD; and Julio Gabriel.

Table. Binding Potential of Carbonyl–Carbon 11–WAY-100635*

<table>
<thead>
<tr>
<th>Brain Area</th>
<th>Patients With JME</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>4.9 ± 0.7†</td>
<td>6.2 ± 0.8</td>
</tr>
<tr>
<td>Motor cortex</td>
<td>2.7 ± 0.6</td>
<td>3.4 ± 1.1</td>
</tr>
<tr>
<td>Amygdala</td>
<td>4.7 ± 0.5</td>
<td>5.1 ± 0.9</td>
</tr>
<tr>
<td>Cingulate cortex</td>
<td>4.1 ± 0.9</td>
<td>4.7 ± 0.8</td>
</tr>
<tr>
<td>Orbitofrontal cortex</td>
<td>3.1 ± 0.5</td>
<td>3.6 ± 0.7</td>
</tr>
<tr>
<td>Insular cortex</td>
<td>4.7 ± 1.0</td>
<td>5.1 ± 1.0</td>
</tr>
<tr>
<td>Dorsolateral prefrontal cortex</td>
<td>3.2 ± 0.5‡</td>
<td>4.1 ± 0.7</td>
</tr>
<tr>
<td>Temporal lateral cortex</td>
<td>4.5 ± 0.6</td>
<td>4.8 ± 0.8</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>3.8 ± 0.7</td>
<td>4.2 ± 0.9</td>
</tr>
<tr>
<td>Raphe nuclei</td>
<td>1.8 ± 0.0‡</td>
<td>2.5 ± 0.4</td>
</tr>
</tbody>
</table>

Abbreviation: JME, juvenile myoclonic epilepsy.
*The binding potential values are given as mean ± SD.
†P < .001.
‡P = .002.
REFERENCES


