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Amygdala Gene Expression of NMDA and GABA<sub>A</sub> Receptors in Patients With Mesial Temporal Lobe Epilepsy

José Carlos de Moura, Daniela P.C. Tirapelli, Luciano Neder, Fabiano P. Saggioro, Americo Ceiki Sakamoto, Tonicarlo R. Velasco, Rodrigo A. Panepucci, João Pereira Leite, João Alberto Assirati Junior, Benedicto Oscar Colli, and Carlos Gilberto Carlotti Júnior

ABSTRACT: Temporal lobe epilepsy (TLE) is the most common form of partial epilepsy and affects 40% of the patients. Seizures arising from the mesial temporal lobe structures (i.e., amygdala and hippocampus) are common, whereas neocortical seizures are rare. In recent years, many studies aimed to identify the pattern of gene expression of neurotransmitters involved in molecular mechanisms of epilepsy. We used real-time PCR to quantify the expression of GABA<sub>A</sub> (subunits α1, β1, β2) and NMDA (subunits NR1, NR2A, and NR2B) receptor genes in amygdalae of 27 patients with TLE and 14 amygdalae from autopsy controls. The NR1 subunit was increased in patients with epilepsy when compared with controls. No differences were found in expression of NMDA subunits NR2A and NR2B or in α1, β1, and β2 subunits of GABA<sub>A</sub> receptors. Our results suggest that the NR1 subunit of NMDA receptors is involved in the amygdala hyperexcitability in some of the patients with TLE.

KEY WORDS: epilepsy; TLE; GABA receptors; glutamate receptors; amygdala

INTRODUCTION

TLE is the most common form of partial epilepsy. It affects 40% of the patients in adult series, and seizures arising from the mesial temporal lobe structures (i.e., amygdala and hippocampus) are the most common type (Babb and Brown, 1986; Hauser, 1992; Bertram, 2009). Several studies have demonstrated that mesial temporal sclerosis (MTS) is the most frequent histological finding in patients with mesial TLE (MTLE), and most of them suggested that cell loss and gliosis in specific subfields of the hippocampus were the most distinctive histological feature (Feuerstein and Hertting, 1986; Andrade and Nicoll, 1987). Most studies using depth electrodes support the notion that the hippocampus is involved in seizure generation in patients with MTLE. However, other studies suggested that the amygdala may also be involved in the epileptogenic process in MTLE (Lindvall et al., 1984; Holland and Gallagher, 1993; Kokaia et al., 1994a,b; Elmer et al., 1996; Bengzon et al., 1999; Aroniadou-Anderjaska et al., 2008; McIntyre and Gilby, 2008). In MTS for example, a reduced volume of amygdala is believed to follow the reduction of the volume of the hippocampus (Bernasconi et al., 2005). Furthermore, studies have shown that up to 30% of seizures in MTLE originate from amygdala (Wennberg, 2002), but not much is known about the mechanisms of hyperexcitability and seizure generation in these patients.

One hypothesis that has been proposed to explain the pathogenic mechanism of epileptogenesis is the imbalance between excitatory and inhibitory neurotransmitters, which has been shown to result in reduced seizure threshold (Neder et al., 2002; Steinhauser and Seifert, 2002; Avanzini and Francescetti, 2003). Glutamate is the major excitatory neurotransmitter (Moldrich et al., 2003), and several studies have shown the role of glutamate receptors, particularly the ionotropic N-methyl-D-aspartate (NMDA) receptor, in epilepsy. In fact, studies in human epileptics have shown an increased expression of NMDA receptor 1 (NMDAR1) transcript in the remaining hippocampal neurons (Mathern et al., 1997; Neder et al., 2002).

We used real-time polymerase chain reaction (q-PCR) to quantify the expression of GABA<sub>A</sub> (α1, β1, β2) and NMDA (NR1, NR2A, and NR2B) subunit genes in 27 amygdalae of patients with temporal lobe epilepsy. Similar to what has been shown in the human hippocampus, we hypothesized that there is an imbalance between excitatory and inhibitory neurotransmission in the amygdalae of patients with...
MTLE, which may contribute to hyperexcitability and seizure generation.

**PATIENTS AND METHODS**

The study was conducted at the University of São Paulo, Ribeirão Preto-Brazil, and patients were enrolled from 2002 to 2004. The follow-up ranged from 42 to 66 months. Patients were considered eligible if they had a medical history, seizure semiology, routine outpatient interictal EEG (electroencephalography), and MRI consistent with refractory MTLE-HS (mesial temporal lobe epilepsy with hippocampal sclerosis). The clinical picture usually consisted of complex partial seizures with epigastric, autonomic, or psychic auras; focal slowing, interictal spikes, and sharp-waves over the anterior, inferior, and mesial temporal regions on routine scalp EEG; and hippocampal atrophy on T1 and increased hippocampal signal on T2 MRI sequences. Refractoriness was defined as failure to respond to at least two antiepileptic drugs. Excluded were patients with extrahippocampal lesions, focal neurological abnormalities on physical examination, and generalized and extratemporal interictal spikes that were not consistent with the MTLE syndrome or that suggested dual pathology.

The samples comprised 27 amygdalae obtained from patients with refractory MTLE submitted to temporal lobectomy and 14 amygdalae obtained from autopsy. The amygdala samples comprised the portion that protrudes to the ventricle cavity, corresponding to the basolateral nucleus. In the autopsy group, no death was caused by neurological or psychiatric disease. The project was approved by the Research Ethics Committee of the University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo.

After removal, the amygdalae were frozen in liquid N$_2$ and kept at $-80^\circ$C until RNA extraction by the Trizol method (Invitrogen, Carlsbad, CA) following the manufacturer’s protocol. The analysis of the controls and patients RNA samples was randomized. All RNA samples were quantified by spectrophotometry and checked in 1% agarose gel. Complementary DNA (cDNA) was synthesized by reverse transcription using the commercial High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, code: 4368814) following the manufacturer’s manual. The cDNA was amplified with q-PCR using SYBR Green (Applied Biosystems). The sequences of the primers for each gene are shown in Table 1.

The β-glucuronidase gene (GUSβ) was used as an endogenous control (housekeeping gene). We used the following PCR conditions: preheating at 50°C for 2 min, denaturation at 95°C for 10 min and 50 cycles of amplification and quantification (15 s at 95°C and 1 min at 60°C). All reactions were carried out in duplicate and analyzed with the 7,500 Sequence Detection System apparatus (Applied Biosystems). The data were analyzed using the ABI-7,500 SDS software. Dissociation curves were performed (melting curves) after amplification by q-PCR. The samples that showed dissociation curves with different temperatures and/or more than one point of dissociation in the same sample were discarded and repeated.

Numerical variables between groups were compared with Student’s t-tests. Significance was set at $P < 0.05$. We used GraphPad PRISM software version 4.0 for statistical analysis (GraphPad Software Inc., San Diego, CA).

**RESULTS**

**Clinical and Demographic Data**

The clinical and demographic data of patients are shown in Table 2. A total of 27 patients were included, and 17 were female (63%). The mean (±SD) age of seizure onset was 6.1 (±5.6) years. The seizure frequency ranged from three seizures a day to one seizure every two months. Eighty-five percent of patients became seizure-free after surgery.

**Gene Expression**

The expression level of the NMDA NR1 gene was higher in the epileptic group when compared with the autopsy group ($P = 0.021$, Fig. 1). The expression of GABA$_A$ α1, GABA$_A$ β1, GABA$_A$ β2, NMDA NR1, NMDA NR2A, and NMDA NR2B genes are also presented in Figure 1, but no differences were found between groups: GABA$_A$ α1 ($P = 0.8669$), GABA$_A$ β1 ($P = 0.0654$), GABA$_A$ β2 ($P = 0.9042$), NMDA NR2A ($P = 0.6295$), and

**TABLE 1.**

**Primer Sequences for GABA$_A$ α1, GABA$_A$ β1, GABA$_A$ β2, NMDA NR1, NMDA NR2A, and NMDA NR2B**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers forward (-f)</th>
<th>Reverse (-r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA$_A$ α1</td>
<td>ATGACGGCTGTCCCATAGCTT</td>
<td>TGGATCCTCTTCTTGACGAC</td>
</tr>
<tr>
<td>GABA$_A$ β1</td>
<td>CGGAGACCATGTCTATGCTG</td>
<td>TGGACAGATGCTCAAAAGA</td>
</tr>
<tr>
<td>GABA$_A$ β2</td>
<td>TGCTTTCATCCTGATTACC</td>
<td>AGTGGGTGTTGATGTTG</td>
</tr>
<tr>
<td>NMDA NR1</td>
<td>GGTACCTCCTGAAGCTTCTCTTCTT</td>
<td>GTTCACTCAACTACAGCATCA</td>
</tr>
<tr>
<td>NMDA NR2A</td>
<td>ACGCGTGCATCTTCTATGCT</td>
<td>AAGTCTTTGCCATCCTCATATG</td>
</tr>
<tr>
<td>NMDA NR2B</td>
<td>TCTTTGGAGATGGGGGAGATG</td>
<td>CCTGCCATTTGTGTCAATGTCT</td>
</tr>
</tbody>
</table>
NMDA NR2B \( (P = 0.5734) \) (Fig. 1). The mean ± S.D. of expression of the NMDA NR1, NMDA NR2A, NMDA NR2B, GABA\(_A\) \( \alpha_1 \), GABA\(_A\) \( \beta_1 \), and GABA\(_A\) \( \beta_2 \) genes are presented in Table 3.

**Gene Expression and Clinical Data**

The expression levels of the NMDA NR1, NMDA NR2A, NMDA NR2B, GABA\(_A\) \( \alpha_1 \), GABA\(_A\) \( \beta_1 \), and GABA\(_A\) \( \beta_2 \) genes did not correlate with any of the clinical and demographic variables (seizure type, age of seizure onset, seizure frequency, and postsurgical seizure outcome). Detailed results of the expression levels for each variable are shown in Table 4.

**DISCUSSION**

In this study we reported the gene expression levels of the \( \alpha_1 \), \( \beta_1 \), and \( \beta_2 \) subunits of the GABA\(_A\) inhibitory neurotransmitter receptor and the NR1, NR2A, and NR2B subunits of the NMDA excitatory neurotransmitter receptor in human amygdalae of 27 patients with MTLE. We found an increased expression of NR1 mRNA in epileptic patients when compared with controls.

Our results support the notion that temporal lobe seizures are associated with increased expression of NMDA receptors in the amygdala, which may contribute to neuronal hyperexcitability, synchronization, and seizure generation (Mathern et al., 1997).

Animal and human studies in hippocampus corroborate our hypothesis that increased NR1 subunit expression can be related to epileptogenesis (Mathern et al., 1997; Kikuchi et al., 2000). Kikuchi et al. (2000) examined the expression level of NMDA NR1 receptor subunit in the cerebral cortices of amygdaloid-kindled rats by northern blot analysis and observed a significant increase of NR1 mRNA expression in the ipsilateral frontal and temporal cortices four weeks after the last generalized seizure. However, a significant decrease in the expression level of NR1 mRNA was observed in the piriform cortices. Toro et al. (2007) used immunohistochemistry to assess NR1 NMDA receptor subunit levels in hippocampi of patients with...
MTLE and found no changes in epileptic patients with schizophrenia-like psychosis or depression compared with controls. In the same study, the expression of NR1 subunit-containing NMDA receptors was higher in dysphoric and untreated depressed patients with epilepsy. Zhu et al. (2004) observed an increase in the expression of NR1 in the hippocampus of rats with epileptic seizures induced by pentyletetrazole, which suggested that the increased expression of NMDA receptors contributed to changes in neuronal excitability in these rats. In contrast, using a PCR technique, Musshoff et al. (2000) did not find any differences in expression of the NR1 subunit in the hippocampus or cortex of epileptic patients when compared with nonepileptics.

Our findings for the NR2A and NR2B subunits showed no differences between groups. Narita et al. (2000) noted an increased expression of NR1, NR2A, and NR2B subunits in limbic forebrains of mice using a western blotting technique. The different results for NR1 and NR2 can be explained by the fact that the stoichiometry of NMDA receptors is not completely understood, but most authors believe that these receptors are tetramers and incorporate two NR1 and two NR2 subunits of the same or different subtypes. The NR1 subunit has different isoforms, the NR2 subunit has four different isoforms and the final form of the receptor is highly variable. New studies of NMDA receptors considering structural aspects, pharmacology and binding domains will collaborate to elucidate our finding (Paoletti and Neyton, 2007).

The distribution of glutamatergic receptors on amygdalar neurons is not uniform. Sah and Lopez De Armentia (2003) found that synapses in the central nucleus of rats activate NMDA receptors that contain NR1 and NR2B subunits, whereas synapses in the lateral nucleus contain receptors with both NR2A and NR2B subunits. In the lateral amygdala, two distinct morphological classes of neurons have been identified: large pyramidal-like neurons, which are thought to constitute the glutamatergic projection neurons, and smaller spine-sparse neurons, which use GABA.

### TABLE 3.

<table>
<thead>
<tr>
<th>Gene Expression Levels in Epileptic and Control Groups</th>
<th>NMDA NR1</th>
<th>NMDA NR2A</th>
<th>NMDA NR2B</th>
<th>GABA&lt;sub&gt;α&lt;/sub&gt;</th>
<th>GABA&lt;sub&gt;α&lt;/sub&gt;</th>
<th>GABA&lt;sub&gt;β&lt;/sub&gt;</th>
<th>GABA&lt;sub&gt;β&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epileptics</td>
<td>1.42 ± 0.71</td>
<td>1.32 ± 1.12</td>
<td>1.11 ± 0.78</td>
<td>0.97 ± 0.51</td>
<td>1.27 ± 0.71</td>
<td>1.26 ± 0.88</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.82 ± 0.49</td>
<td>1.00 ± 0.83</td>
<td>1.14 ± 0.88</td>
<td>1.03 ± 0.71</td>
<td>0.97 ± 0.54</td>
<td>1.26 ± 0.89</td>
<td></td>
</tr>
</tbody>
</table>

Values are shown as mean ± SD.
### TABLE 4. Expression Levels According to Clinical Data

<table>
<thead>
<tr>
<th>Seizure type</th>
<th>Age of onset</th>
<th>Seizure frequency</th>
<th>ENGEL classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial</td>
<td>10 years</td>
<td>&gt;5 Seizures/week</td>
<td>I and II</td>
</tr>
<tr>
<td>Generalized</td>
<td>&gt;10 years</td>
<td>≤5 Seizures/week</td>
<td>III and IV</td>
</tr>
<tr>
<td></td>
<td>≤10 years</td>
<td>≥5 Seizures/week</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>NMDA NR1</th>
<th>NMDA NR2B</th>
<th>GABAA A1</th>
<th>GABAA A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial</td>
<td>1.57 ± 0.29</td>
<td>0.93 ± 0.34</td>
<td>0.49 ± 0.12</td>
<td>1.25 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>1.32 ± 0.25</td>
<td>0.91 ± 0.18</td>
<td>0.59 ± 0.08</td>
<td>1.12 ± 0.08</td>
</tr>
<tr>
<td>Generalized</td>
<td>1.32 ± 0.25</td>
<td>0.91 ± 0.18</td>
<td>0.59 ± 0.08</td>
<td>1.12 ± 0.08</td>
</tr>
</tbody>
</table>

Values are shown as mean ± SD.

as the primary transmitter. We could not make these inferences in the present study because real-time PCR does not permit the cellular localization of neurotransmitter expression.

We did not find differences in the expression of α1, β1, or β2 subunits of GABA<sub>A</sub> receptors between groups. Generally, the studies on the role of GABA<sub>A</sub> receptors in MTLE have utilized immunohistochemistry and focused on the hippocampus. Boullieret et al. (2000) investigated alterations in subunit architecture and localization of GABA<sub>A</sub> receptor subtypes immunohistochemically (α1, α2, α3, β2, β3, and γ2) in the hippocampus resected from MTLE patients compared with control tissue obtained at autopsy. They found changes in GABA<sub>A</sub> receptors with reorganization of specific receptor subtypes in some cells and interneurons. In addition, Crino et al. (2001) found different patterns of GABA<sub>A</sub> receptor subunit expression in cortical dysplasia. Using immunohistochemistry, the authors observed a decrease in the expression of the β1 subunit in dysplastic neurons compared with pyramidal and heterotopic neurons. They also observed a reduction in the expression of GABA<sub>A</sub> receptor α1, α2, and β2 subunits in both dysplastic and heterotrophic neurons.

Ion channel dysfunction is the key to the genetic human epilepsies. There is increasing evidence that changes in ion channels form a common pathway for diverse causes of epilepsies (Berkovic et al., 2006).

Our results support the notion that there are changes in amygdala glutamate receptors in some patients with mesial temporal lobe seizures, which may be associated with increased neuronal hyperexcitability, synchronization, and seizures. New studies are needed to identify those patients with MRI findings to determine whether surgery could be tailored in response to the need for the amygdala to be resected.

### REFERENCES


AMYGDALAR EXPRESSION OF NMDA AND GABA_A IN PATIENTS WITH MTLE


