ORIGINAL ARTICLE

Changes in functional coupling of 5-HT\textsubscript{1A} receptor to the G-protein in neocortex temporal tissues of patients with temporal lobe epilepsy

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KEYWORDS
5-HT\textsubscript{1A} receptor; G-protein; Lateral temporal neocortex; Mesial temporal lobe epilepsy

Abstract

\textbf{Background:} The 5-hydroxytryptamine-1A receptors are known to be involved in the inhibition of seizures in epilepsy.

\textbf{Objective:} The main aim of the present study is to determine the functional coupling to G-protein of the 5-HT\textsubscript{1A} receptor through 8-OH-DPAT-stimulated \textsuperscript{35}S\textsuperscript{GTP\textsubscript{S}} binding assay in lateral temporal neocortex tissue of patients with mesial temporal lobe epilepsy (mTLE) and correlate it with clinical data.

\textbf{Material and methods:} The activation of the G protein complex was determined by \textsuperscript{35}S\textsuperscript{GTP\textsubscript{S}} binding assay. The temporal neocortex tissue was obtained from 5 patients with mTLE during epilepsy surgery and from 5 subjects (autopsies) who died due to an accident and without history of neurological disease.

\textbf{Results:} We found that values of maximal stimulation (E\textsubscript{max}) of \textsuperscript{35}S\textsuperscript{GTP\textsubscript{S}} binding significant decreased in the mTLE group (30.8%, \(p<.05\)) when compared to the autopsy samples (65.9%). The values of potency (pEC\textsubscript{50}) were similar in both groups. However, we found no significant differences between the E\textsubscript{max} and age of patient, age of seizure onset, duration of epilepsy and frequency of seizures. Also, the pEC\textsubscript{50} values revealed no significant correlations with the clinical data.

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Conclusions: Our data provide evidence that the lateral temporal neocortex of patients with pharmaco-resistant mTLE presents alterations in the functional coupling to G-protein of the 5-HT₁A receptor. Such a change could be involved in hyperexcitability in the neocortex of patients with mTLE.

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Introduction

The G-protein-coupled receptors (GPCRs) are membrane proteins that sense signaling molecules such as hormones, light, odors, taste and neurotransmitters.¹ GPCRs undergo conformational changes after ligand binding, causing the activation of signaling networks, resulting in a cellular response. G proteins are heterotrimeric made of α, β and γ subunit. When the receptor stimulates the G protein, the α subunit releases guanosine diphosphate (GDP) and binds to GTP (guanosine triphosphate). The Gα subunits are divided in Gα₁, Gα₁₂/₁₃, Gα₁₃, and Gα₁₅ based on sequence homology and functional similarities, each one active a cascade of second messengers¹ (Fig. 1). The Gα₁₅ subunit is interest, because it inhibits adenylyl cyclases (AC) and reduces the concentration of cyclic adenosine monophosphate (cAMP), this leads to mediate receptor-dependent inhibition of various types of adenylyl cyclases, in addition to being expressed more in brain.²

The metabotropic receptors Gαι/γ-protein-coupled have been considered as promising drug targets in the treatment of several disease, related to excitation disorders such as epilepsy.

Epilepsy is a chronic neurological disorder characterized by increased excessive or synchronous abnormal neuronal activity in the brain.³ Numerous studies imply an imbalance between excitatory and inhibitory neurotransmission.³ The neurobiological basis of epilepsy involves alterations of neuronal functions at multiple levels. It is known that changes in neurotransmission systems as receptors coupled to G-proteins are involved in epileptic seizures.⁴⁻⁸ The activation of GPCR receptors specifically those coupled to Gαι/γ proteins have demonstrated anticonvulsive, antiepileptic and neuroprotective effects in different experimental models of epilepsy.⁹⁻¹² In contrast, brain tissue of patients with epilepsy showed alterations in their density and functional activity.¹³⁻¹⁵ These evidences in preclinical models and brain tissue of patients with epilepsy suggest the participation of receptors to Gαι/γ proteins in epilepsy.

Cambiós en el acoplamiento funcional del receptor 5-HT₁A a la proteína-G en tejido de neocorteza temporal de pacientes con epilepsia del lóbulo temporal

Resumen

Antecedentes: Los receptores 5-hidroxitriptamina-1A (5-HT₁A) están implicados en la inhibición de las crisis en epilepsia.

Objetivo: El presente estudio es determinar el acoplamiento funcional a la proteína-G del receptor 5-HT₁A a través del ensayo de unión [³⁵S]GTPγS estimulando al receptor por 8-OH-DPAT (agonista del receptor 5-HT₁A) en tejido de neocorteza temporal de pacientes con epilepsia del lóbulo temporal mesial (ELTm) y correlacionar con datos clínicos.

Material y métodos: La activación del complejo de la proteína G del receptor de 5-HT₁A se determinó por [³⁵S]GTPγS por ensayos de unión. Tejido de neocorteza temporal de pacientes con ELTm obtenido de la cirugía de epilepsia y 5 tejidos de autopsias de sujetos que fallecieron por accidente o por causas diferentes a una enfermedad neurológica.

Resultados: Los resultados demostraron estimulación máxima (Eₘₐₓ) significativamente disminuida en el grupo ELTm (30.8%, p < 0.05), en comparación con tejido de muestras de autopsia (65.9%). Los valores de potencia (pEC₅₀) fueron similares en ambos grupos. Sin embargo, no se encontraron diferencias significativas entre la Eₘₐₓ con la edad del paciente, la edad de inicio de las crisis, duración con epilepsia y la frecuencia de crisis. Además, los valores de pEC₅₀ no revelaron correlación con los datos clínicos.

Conclusión: Los presente estudio proporcionan evidencia que la neocorteza temporal de pacientes con ELTm fármaco resistente presenta alteraciones en el acoplamiento funcional a la proteína-G del receptor 5-HT₁A. Tal cambio podría estar implicado en la hipereexcitabilidad que se observa en la neocorteza de pacientes con ELTm.

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Changes in functional coupling of 5-HT1A receptor

The functional activity of the 5-HT1A receptor is coupled to G-proteins and involves several downstream targets. One of the key targets is the adenylate cyclase, which is activated by the binding of G-protein coupled to the receptor. This activation leads to the production of cyclic adenosine monophosphate (cAMP), which in turn activates protein kinase A (PKA). The PKA then phosphorylates various target proteins, leading to changes in cell function.

The functional activity of the 5-HT1A receptor can also be modulated by the presence of other neurotransmitters and neuromodulators. For example, the binding of serotonin to the 5-HT1A receptor can increase the activity of the G-protein coupled to the receptor, leading to an increase in cAMP production. This can be mediated by the activation of other G-protein coupled receptors, such as the 5-HT3A receptor.

However, the functional activity of the 5-HT1A receptor can also be inhibited by the binding of other neurotransmitters, such as acetylcholine. This inhibition can be mediated by the activation of G-protein coupled receptors, such as the M1 muscarinic receptor, which can inhibit the activity of the G-protein coupled to the 5-HT1A receptor.

In conclusion, the functional activity of the 5-HT1A receptor is influenced by a variety of factors, including the presence of other neurotransmitters and neuromodulators, and the binding of agonists and antagonists to the receptor. This complex interplay between different factors is crucial for understanding the functional activity of the 5-HT1A receptor in different physiological and pathological conditions.

Figure 1  Representative image of the G-protein-coupled receptors (GPCRs) associated with the different Gα subunits (Gαi5, Gαi1, and Gα12/13). AMPc: cyclic adenosine monophosphate; DAG: diacylglycerol; IP3: inositol triphosphate; +: stimulate; −: inhibit.
without evidence of neurological disease. The control autopsy samples were prepared by the Institute of Forensic Sciences, in agreement with the Faculty of Medicine of the National Autonomous University of Mexico. Our tissue was dissected at the time of autopsy, with a postmortem interval of 3 to 6 h, immediately stored at −70 °C and then manipulated as described below.

**Binding assays**

**Membrane preparation**

The lateral temporal neocortex tissues were prepared according to the method previously described\(^\text{27}\) slight modifications. Briefly, samples (200–500 mg) were homogenized on ice in 50 mM Tris–HCl buffer (pH 7.4) using a Teflon-glass homogenizer. The homogenate was centrifuged at 15,000 rpm for 25 min at 4 °C and the resulting pellet was resuspended in fresh buffer and incubated for 30 min at 35 °C. The centrifugation step was repeated, and the final pellet was re-suspended in 50 mM Tris–HCl buffer (pH 7.4) and stored at −70 °C until use. Protein levels were determined by the method of Lowry et al. (1951).\(^\text{28}\)

\[^{35}S\]GTP\(_\gamma\)S functional assay

The 5-HT\(_{1\alpha}\) receptor-mediated G-protein activation experiments were carried as previously described for Spetea et al. (1998)\(^\text{29}\) with slight modifications. The membrane fractions (≈10 μg of protein/sample) of lateral neocortex temporal tissue were incubated at 30 °C for 60 min in Tris–EGTA buffer (1 M Tris–HCl, 0.2 M EGTA, 1 M MgCl\(_2\), 1 M NaCl, pH 7.4), containing \([^{35}S]\)GTP\(_\gamma\)S (0.05 nM) and increasing concentrations (Log 10\(^{-11}\) to 10\(^{-5}\) M) of 8-OH-DPAT (5-HT\(_{1\alpha}\) receptor agonist) in presence of excess GDP (10 mM) in a final volume of 1 ml. Total binding was measured in absence of the tested compound. Non-specific binding was determined in presence of 10 mM unlabeled GTP\(_\gamma\)S and subtracted from total binding to calculate the specific binding. The reaction was initiated by adding \([^{35}S]\)GTP\(_\gamma\)S and completed with filtration of the samples through Whatman GF/B glass fiber filters; it was washed three times with ice-cold 50 mM Tris–HCl buffer (pH 7.4) using Brandel M-48 Cell Harvester. The dried filters were immersed in a Sigma-FluorTM scintillation cocktail (Sigma) and then used to determine bound radioactivity as described above. The binding experiments were performed in triplicates.

**Data analysis**

The data were expressed as mean ± S.E.M for the subject's age, age of seizure onset, duration of epilepsy and frequency of seizures. G-protein activation was given as percent of the specific \([^{35}S]\)GTP\(_\gamma\)S binding observed in absence of receptor ligands (basal activity). The binding data were subjected to non-linear regression analysis of concentration-effect curves performed by Prism (GraphPad Software, Inc.) for determined agonist potency (pEC\(_{50}\)) and the maximum stimulation (E\(_{max}\)) values. The data were expressed as mean ± S.E.M. The results from the specific binding were examined statistically by Student’s t-test to find differences of E\(_{max}\) and pEC\(_{50}\) between autopsy samples and mTLE tissue. Pearson’s correlation coefficients were calculated to establish a significant
Changes in functional coupling of 5-HT1A receptor

![Graph showing binding of [35S]GTPγS](image_url)

Figure 2. Specific [35S]GTPγS binding to membranes of samples from autopsies (■) and from patients with mTLE (○) as a function of increasing concentration of the 5-HT1A receptor agonist 8-OH-DPAT. Each point represents the mean ± S.E.M of the percentage of stimulation over basal values. Note that in patients with mTLE, [35S]GTPγS binding stimulation by 8-OH-DPAT was significantly different from that of autopsy samples. correlation (p < 0.05) of $E_{\text{max}}$ and $pEC_{50}$ with the obtained values of patient’s age, age of seizure onset, duration of epilepsy and frequency of seizures.

Results

Autopsy samples

Autopsy samples were acquired from five subjects (two male and three female) ranging in age from 29 to 71 years (45.8 ± 7.2 years), who died by different causes and without history of neurological disease. The postmortem interval was 3 to 6 h (4.6 ± 0.5 h) (Table 1). 8-OH-DPAT-stimulated [35S]GTPγS binding was concentration-dependent and saturable. The binding assays revealed $E_{\text{max}}$ of 65.9% and a potency $pEC_{50}$ value within the micromolar range (−7.3 ± 0.3) (Fig. 2). We found no significant correlation in autopsy samples between the coefficients of the $E_{\text{max}}$ of [35S]GTPγS binding and $pEC_{50}$ values, with the age ($r = 0.7453$, $r = 0.0989$, respectively) and postmortem interval ($r = 0.7092$, $r = 0.0305$, respectively) (Table 2).

Patients group

The lateral temporal neocortex tissues were obtained from five patients (two male and three female) of age 38.2 ± 2.9 years old (ranged from 29 to 45 years) with 25.6 ± 2.6 years of epilepsy duration; the age of seizure onset was 12.6 ± 3.3 years and they had 22.8 ± 7.5 seizures per month (Table 1). We found no significant differences in age between mTLE patients and the autopsy samples (38.2 ± 2.9 and 45.8 ± 7.2 years, respectively, $p = 0.1766$). Therefore, we found that $E_{\text{max}}$ values significantly decreased in the mTLE group (30.8%, $p < 0.05$) when compared to autopsy samples (65.9%) (Fig. 2). However, the $pEC_{50}$ values in the micro molar range were similar in both groups (−7.3 ± 0.3 and −7.2 ± 0.1, autopsy samples and mTLE, respectively). Regarding the correlation analysis, we found no significant differences between the $E_{\text{max}}$ with age of patient, age of seizure onset, duration of epilepsy and frequency of seizures. Also, the $pEC_{50}$ values revealed no significant correlations with clinical data (Table 2).

Discussion

Even though the results showed a decrease of the 5-HT1A receptor-induced G-protein functional activation in mTLE, we found no correlation between the decrease in functional coupling to the 5-HT1A receptor with the patient’s age, duration with epilepsy, age at onset with epilepsy, and frequency of seizures.

In the present study, we find limitations that describe below. Previous studies have indicated that 5-HT1A receptor density decreases with age in brain regions such as cortex, hippocampal regions, locus coeruleus and dorsal raphe nuclei.10,31 These results suggest that a decrease in receptor density could indirectly produce changes in functional 5-HT1A receptor activation. An important point to mention, we used tissue from mTLE surgical patients and autopsy samples from subjects of similar age, in order to reduce the possible influence of this variable. Also, our results show no significant differences in age between mTLE patients and the autopsy samples neither correlation with 5-HT1A receptor-induced G-protein functional activation. Our results suggest that decrease functional 5-HT1A receptor activation is directly related to changes associated with epilepsy rather than age.

Concerning the postmortem interval of the autopsy samples (4.6 ± 0.5 h) may produce changes that could alter the results, in this context, previous studies indicate that 5-HT1A receptors are preserved within a postmortem interval of 8–92 h.32 Also, we results showed that postmortem interval did not correlate with the 5-HT1A receptor-induced G-protein functional activation. These previous studies and

Table 2. Correlations of [35S]GTPγS binding parameters between clinical data and data from the autopsy sample from neocortex temporal membranes in patients with temporal lobe epilepsy.

<table>
<thead>
<tr>
<th>[35S]GTPγS binding assay</th>
<th>Clinical data</th>
<th>Data from the autopsy samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age of patient</td>
<td>Seizure onset age</td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>0.3906</td>
<td>−0.0991</td>
</tr>
<tr>
<td>$pEC_{50}$</td>
<td>−0.6604</td>
<td>−0.0280</td>
</tr>
</tbody>
</table>

Values represent Pearson Correlation Coefficients. $E_{\text{max}}$ values (expressed as maximal stimulation of [35S]GTPγS binding) and $pEC_{50}$ values (expressed as the negative logarithm of EC50).
results obtained lead us to suggest that tissue from autopsy samples can be used as control.

Another limitation was the fabric storage time at −70 °C. It has been reported that storage intervals of 1–82 months do not affect the functional activity of the 5-HT1A receptor. In our report the storage time was 4–12 months.

Regarding our results that showed a decrease in functional 5-HT1A receptor activation in patients with mTLE can be explained as a consequence of changes in density of receptor in neocortex. It is known that the cortical layers (I–II) of the neocortex present a dense pattern of these receptors. Rocha et al. (2007) showed decreased of 5-HT1A receptor binding in the external cortical layers (I–II) of patients with mTLE. Other studies with PET have shown decrease the 5-HT1A receptor binding in neocortex of patient with mTLE. These evidences may partly explain the decrease in the receptors functional activity in our results.

It is important to mention that 5-HT1A receptor-induced G-protein functional activation induces hyperpolarization via potassium channels and therefore inhibitory effects. Therefore, the decreased functional activity of 5-HT1A receptors associated with reduced density of these receptors in neocortex; it could explain the excitability and facilitation of seizure activity in neocortex of patients with mTLE. On the contrary, study in epileptic hippocampal tissue show an increase of the 5-HT1A receptor-induced G-protein functional activation suggest as a compensatory mechanism in response to the epileptic discharges that could contribute to the modulation of neuronal hyperexcitability.

The results of the present study indicate that patients with mTLE do not observe correlation with clinical aspects. These results suggest that clinical aspects do not play an important role in changes in the attachment of Gαi/o proteins to 5-HT1A receptors. However, we cannot rule out other factors such as antiepileptic drugs, cortical atrophy or other processes underlying epilepsy may be involved in the observed alterations in functional activity.

The development of drugs that target the activation of GPCR receptors specifically those coupled to Gαi/o proteins has recently gained new interest for its efficacy in preclinical models by their anticonvulsive, antiepileptic, and neuroprotective effects. However, it is important to consider that at present is insufficient evidence of the alterations in coupled to Gαi/o proteins have in pharmacoresistance in patients with epilepsy. These studies in surgically resected tissue of patients with pharmacoresistant epilepsy will allow us to investigate and provide the scientific basis for these alterations in epilepsy, thus leading to know whether they can be used as a therapeutic target to treat epilepsy in the future.

Conflict of interest statement

None of the authors has any conflict of interest to disclose.

References

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