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Abstract

Epilepsy is a chronic neurological condition characterized by abnormal brain activity, unusual behavior, and loss of awareness. One of the most common features is the spontaneous recurrence of unprovoked seizures that mainly affect the hippocampus and cortical regions of the brain. Although the exact cause of epilepsy is still unknown, a mix of genetic, neurological, and environmental factors play a role. A novel study by Yang et al. explores the metabotropic receptor GPR40 which is suspected to be involved in the regulation of epileptic seizures, specifically through its modulatory role on NMDA receptors in the central nervous system. Their findings suggest that GPR40 induces NMDA receptor endocytosis via direct interaction with NR2A and NR2B subunits of postsynaptic NMDA receptors. Through this mechanism, NMDA-mediated postsynaptic currents are altered, resulting in reduced seizure-like activity. This review article discusses these novel findings which not only shed light on the potential molecular mechanisms of epilepsy but also push the scientific community closer to developing a treatment for this disorder.

Keywords: Epilepsy, GPR40, seizures, NMDA receptors, NR2A and NR2B subunits, excitatory postsynaptic currents, cortex, and hippocampus

1. Introduction

Epilepsy is a chronic neurological condition that affects an estimated one to three percent of the population; it is characterized by the spontaneous recurrence of unprovoked seizures, seizures that occur without apparent triggers.[1] Epilepsy can also be characterized by abnormal brain activity, unusual behavior or sensations, and even a loss of awareness, though these are often common characteristics of other neurological disorders as well.[2] Other general symptoms of epilepsy may include temporary confusion, uncontrollable motor function, loss of consciousness, and even psychological symptoms, such as anxiety, fear, or deja vu.[2]

The exact cause of epilepsy has not yet been determined. However, the neurological disorder has been connected to a variety of factors.[2] The onset of epilepsy has been associated with several developmental disorders including autism and neurofibromatosis. It has also been associated with infectious diseases such as meningitis, acquired immunodeficiency syndrome, and viral encephalitis. Brain trauma caused by stroke or brain tumors can also lead to the onset of epilepsy; in fact, stroke is known to be a common cause of epilepsy in individuals over thirty-five years old.[2]

Additionally, head trauma and traumatic brain injury can play a role in the development of epilepsy. Lastly, research has supported links between epilepsy and specific genes in the human genome. Some genes may contribute to making an individual more susceptible to environmental conditions that might trigger seizures, making genetic influence another factor in the development of epilepsy.[2]

The relationship between genetic influence, gene expression, and protein synthesis has shed light on a new protein, G-protein coupled receptor 40 (GPR40), that is involved in the regulation of epileptic seizures, specifically through its role in regulating NMDA receptors in the central nervous system.[3] The novel study conducted by Yang et al, "GPR40 modulates epileptic seizure and NMDA receptor function," investigated the impact of GPR40 on epileptic brains, and more specifically, how expression of GPR40 affected NMDA receptor function and NMDA receptor-mediated synaptic transmission. This study determined the specific localization of GPR40 in the brain and central nervous system, how the upregulation of the receptor affects epileptic seizures, and how NMDA receptor mediated synaptic transmission function is affected, through regulation of specific subunits of the NMDA receptor on the postsynaptic neuron.[3]

Various antiepileptic drugs (AEDs) have been synthesized to combat seizure activity in epileptic individuals. A meta-analysis reviewing the efficacy of 11 antiepileptic drugs administered to over 900 patients revealed that pregabalin, tiagabine, and vigabatrin yielded the most significant reductions in seizures (>50%).[5] However, similar meta-studies have revealed alternative drugs as the most effective, taking factors such as adverse side effects and tolerability of the drugs into account. While many of these AEDs have adequate efficacy, 30% of patients using these drugs continue to experience some side effects of the treatment, including insomnia, depression, and dizziness.[3] These antiepileptic drugs target neurotransmission, specifically to maintain the balance of excitatory and inhibitory neurotransmission that is normally disrupted in epileptic brains.[3] For the development of a more effective antiepileptic drug that can both treat and limit the side effects of epileptic seizures, many factors must be taken into account, including the neurological root of the disorder. The investigation conducted by Yang et al. aims to obtain a deeper understanding of the root cause of epileptic seizures and the onset of epilepsy.[3] These studies may contribute to the future development of a more effective cure for this chronic neurological condition.

As aforementioned, epilepsy is characterized by excessive and abnormal neuronal activity. The precise mechanism is yet to be discovered as epilepsy is known to have multiple pathophysiological causes including genetic mutation, traumatic brain injury, and exposure to toxins. However, the imbalance of excitatory and inhibitory neurotransmitters is understood as the most common mechanism of various epileptic seizures.[6] To elaborate, seizures are often the result of sudden synchronized neuronal signaling and changes in inhibitory and excitatory stimulation. Many neurotransmitters and hormones such as serotonin, norepinephrine, histamine, are involved in epilepsy.[7] Gamma-aminobutyric acid (GABA) and nicotinic acetylcholine (ACh) receptors, in particular, have been implicated. Mutations in GABA receptors have been shown to cause ER stress and ion imbalance, contributing to epileptogenesis. Additionally, dysfunctional acetylcholine signaling has been shown to aggravate inflammation, a key feature of epilepsy.[7]

Although epilepsy can occur in any region of the brain, it is known to affect the frontal lobe and the temporal lobe most commonly, particularly the hippocampus.[8] Therefore, studying the regulatory mechanisms of neurotransmitters in cortical and hippocampal regions of the brain is important in furthering our understanding of epilepsy. Interestingly, recent studies have found that GPR40 plays a role in modulating synaptic transmission, especially in the cortex and hippocampus.[3] Therefore, the Yang et al. study

was set out to investigate the role of GPR40 in epileptic seizure.[3]

2. Description of GPR40 Involvement in Regions of the Brain

While previous studies have shown that GPR40 is expressed in the cortex and hippocampus, the precise distribution and expression levels are poorly understood.[9] The novel study by Yang et al., demonstrated the distribution and expression levels of GPR40 in both normal and epileptic rodent brains through immunofluorescence staining.[3] This methodology revealed that GPR40 was highly expressed in the lacunosum moleculare layer and the pyramidal cell layer of the hippocampus, while it was not highly expressed in the dentate gyrus. The study further evaluated the cellular level localization of GPR40 from a kainic acid (KA) induced temporal lobe epilepsy (TLE) model and normal control brain. GPR40 was colocalized with microtubule-associated protein 2 (MAP2; a marker of dendrites) and postsynaptic density-95 (PSD95; a postsynaptic marker) but not with glial fibrillary acidic protein (GFAP; an astrocyte marker) in the hippocampus of both the epileptic brain and normal control brain.[3] These results suggested that GPR40 is mostly expressed in postsynaptic excitatory neurons but not in astrocytes.[3]

Expression levels of GPR40 in the hippocampus of epileptic and nonepileptic tissues were compared to validate that GRP40 expression is meaningfully correlated with epilepsy. Immunofluorescence signals of GPR40 were significantly increased in the CA1 hippocampal region of the epileptic rodent brain model compared to those of the nonepileptic control mice. Consistent with this mouse model, high GPR40 expression was observed in the human neocortex of TLE patients. In the study, the team conducted a western blot experiment to further validate that GPR40 protein expression increased in epileptic rodent brain models compared to normal control brains. Congruent with the previous results, the epileptic brain models showed significantly higher GPR40 expression in the cortex and hippocampus compared to the control.[3] Elevated GPR40 levels in epileptic brains suggest a possible role of GPR40 in modulating epilepsy.

Therefore, the experiments suggest that GPR40 may be involved in epilepsy. However, further experiments must be conducted in order to discover the direct relationship between GPR40 and epilepsy. If GPR40 directly downregulates or upregulates epileptic seizures, then it is crucial to further study its regulatory mechanisms and signaling pathway.

3. NMDAR-Mediated GPR40 Signaling Modulates Epileptic Seizures

Also known as the Free Fatty Acid Receptor 1, GPR40 is bound and activated by long, unsaturated fatty acids called PUFAs. The existing literature has argued that PUFAs may have differential effects in the CNS.[10, 11] In fact, one group in particular, omega-3 PUFAs, has been shown to have anticonvulsant effects. A study by the University of Toronto's Epilepsy Research Program found that various fatty acid chains, including ALA, EPA, and DHA, reduced the frequency of action potentials and excitatory signaling *in vitro*.[10] However, they posited that the observed effects were modulated through voltage-gated Na^+ and Ca^{2+} channels, further supporting that seizure-like activity is dependent on a plethora of signaling pathways and factors. In contrast, other studies present confounding results, showing that low doses of certain PUFAs reduce seizure frequency whereas increased doses have no significant effects on seizure frequency.[11] PUFAs may therefore act on many target locations, either reducing or increasing seizure-like activity. These conflicting results reveal the need for further research to fully understand the various, complex factors that modulate epileptic activity.

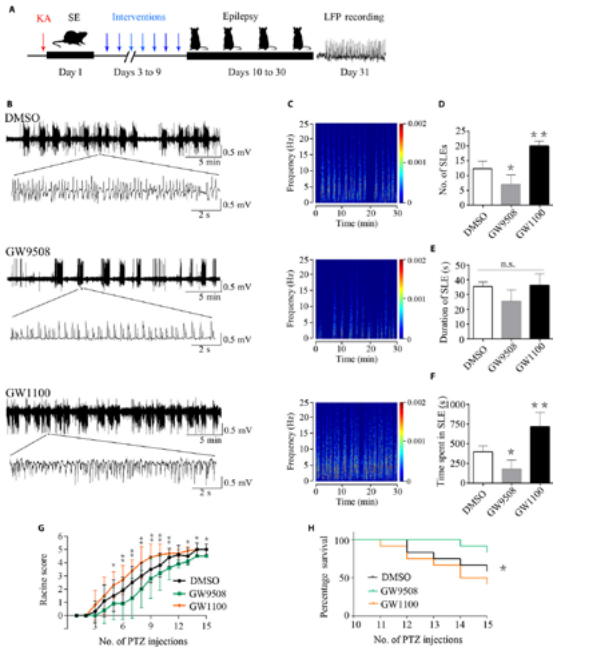

Figure 1: Activation of GPR40 via KA-induced and PTZ kindling models Yields Highest Survival Rates. a) Representation of KA-induced experimental timeline. After KA injection, mice were treated with DMSO, GW9508, or GW1100 (n=6 in each group). b) LFP recordings from each treatment group over 5 minutes and zoomed in of 2 s. c) Corresponding frequency recordings of LFP. d-f) Comparing three groups in number, duration, and time spent in SLEs. g) Using the PTZ model, seizure activity was heightened in GW1100 and reduced in GW9508 groups. h) Highest survival amongst GW9508 group and lowest survival amongst GW1100 group (Figure taken from Yang et al., 2018).

Yang et al. discovered that GPR40 activation results in a layered cascade of signaling events. Overall, GPR40 affected NMDA receptor endocytosis by binding to NR2A and NR2B subunits found on neuronal membranes. To elaborate, the research team needed to confirm that GPR40 played a direct, causal role in modulating epileptic seizures. In order to accomplish this initial goal, they utilized an intrahippocampal KA-induced TLE model, meaning that KA was injected into mice's hippocampi unilaterally to induce seizures (Figure 1A). Previous studies have shown that KA, an excitatory amino acid, is a useful tool for seizure-induction because it induces certain seizures that are commonly experienced by patients with temporal lobe epilepsy.[12] Three days after the induction of epileptic activity, additional compounds were injected daily for one week. These treatments included a DMSO control, GW9508 agonist, or GW1100 antagonist of the target GPR40 receptor. After one-month, local field potentials, which were characterized as strong electrical signals between neurons, were measured (Figure 1B). These measurements reflected both the frequency and duration of any seizure-like events (SLE's) experienced by the mice. The compiled data revealed that mice injected with the GW1100 antagonist experienced more seizure-like events that lasted longer compared to the control (Figure 1D, F). In contrast, mice injected with the GW9508 agonist experienced fewer seizure-like events that lasted a significantly shorter time compared to the control (Figure 1D, F).[3]

To confirm their findings, the researchers repeated their experiment with a pentylenetetrazol (PTZ) kindling model. Like kainic acid, pentylenetetrazol, a GABA A receptor antagonist, was used to induce convulsive activity in the mice[13]; this model therefore represents the seizure-like symptoms experienced by many patients suffering with epilepsy. Like the previous model, the mice received intracerebroventricular injections of one of three treatments: the DMSO control, the GW9508 agonist, or the GW1100 antagonist. While all three mice groups showed increased seizure scores after PTZ treatment, the mice treated with GPR40 selective agonist exhibited significantly lower seizure scores compared to those treated with GPR40 antagonist. As PTZ injections continued to be administered, the mice treated with the agonist had the highest survival rate, while the mice treated with the antagonist had the lowest survival rate and were most prone to generalized tonic-clonic seizure (GTCS) related death (Figure 1G, H). These findings served as a key indication that activating or inhibiting GPR40 receptors directly affects epileptic seizure activity; specifically, activating the receptor resulted in reduced epileptic activity and an increased chance

for survival, while inhibiting the receptor led to opposite observed effects.[3]

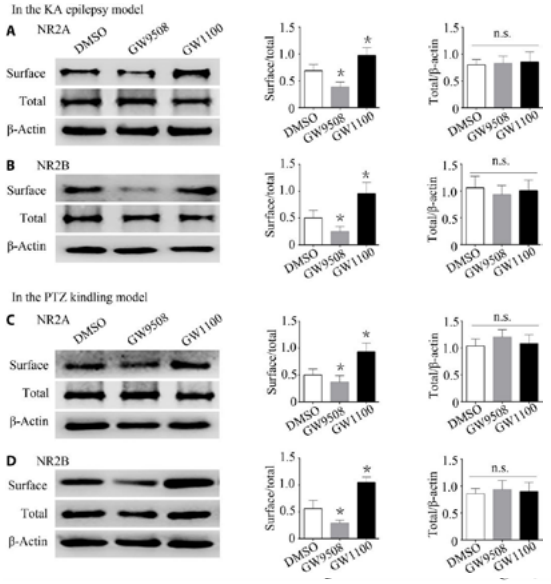

Figure 2: Changes in Cell Surface NR2A and NR2B Subunit Expression in Hippocampal Tissue Samples in GPR40-treated Mice during Epilepsy. After KA injection or PTZ treatment, mice were treated with DMSO, GW9508, or GW1100 (n=5 in each group). a-b) KA model: cell surface and total N2RA and NR2B expression was quantified via western blot. c-d) PTZ model: cell surface and total NR2A and NR2B expression was quantified via western blot. Significant differences were observed in both treatment models between antagonist and agonist groups. One-way ANOVA and Tukey's Test (Figure taken from Yang et al., 2018).

The next aim was to determine how GPR40 regulated seizure-like activity. They found that GPR40 regulated the functions of NR2A and NR2B as well as NMDAR-mediated synaptic responses. Interestingly, the different subunits of the NMDA receptor are referred to as a heterotetrameric assembly. Specifically, the role of the NR2 subunit in epilepsy was evaluated by determining the impact of GPR40 on NMDAR regulation (Figure 2).

In order to study the regulation and cell surface expression of NR2 subunits, the experimenters treated mice with KA and PTZ, then harnessed hippocampal slices from these mouse models. They quantified total expression and cell surface expression of both NR2A and NR2B subunits for the GW9508, GW1100, and DMSO groups and compared them against a standard control of b-actin. It was found that when compared to the DMSO group, NR2A/B showed no significant change in total expression for both the GW9508 and GW1100 models (Figure 2). However, the ratio of surface to total expression of both NR2A and NR2B was significantly reduced for the GW9508 agonist treatment group whereas the opposite results were observed for the GW1100 antagonist treatment group. (Figure 2). These results suggest that GPR40 impacted the cell surface expression of both NMDA subunits in the hippocampal tissues.

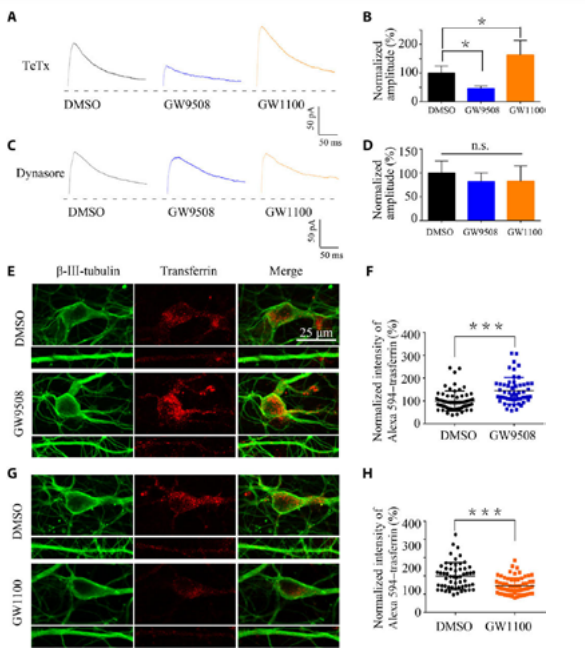

Figure 3. GPR40 Regulates NR2A and NR2B Endocytosis, thereby Affecting NMDA-mediated Postsynaptic Currents. CA1 Hippocampal neurons were isolated from brain tissue treated with endocytosis and exocytosis blockers. a-d) Measured NMDAR-EPSCs after treatment with 0.1 M TeTx or 80 mM dynasore (n=5 in each group). Significant difference (*P < 0.05) observed between control and treatment groups when treated with TeTx. One-way ANOVA and Tukey's Test. e-f) Confocal image and analysis showing differences in Alexa 594-transferrin uptake between 20 M GW9508 and DMSO treatment groups. g-h) Confocal image and analysis showing differences in Alexa 594-transferrin uptake between 20 M GW1100 and DMSO treatment groups (Figure taken from Yang et al., 2018).

This finding was further analyzed, shedding light on the regulation of GPR40 on the surface level expression of NMDARs. This was done through the examination of NMDA-EPSCs (NMDA-excitatory postsynaptic currents) on specific CA1 hippocampal neurons that were obtained from brain tissue slices treated with endocytosis and exocytosis blockers. Interestingly, NMDA-EPSCs amplitudes were decreased in GW9508 but increased in GW1100 (Figure 3B). However, this effect was not seen in the presence of the endocytosis blocker dynasore, suggesting that GPR40 regulates MNDA transmission through endocytic mechanism (Figure 3B, D).

In order to quantify the effect of GRP40 on endocytosis, an Alexa-594-transferrin uptake assay of cultured neurons that had been previously treated with GW9508 and GW1100 was used. GW9508 showed an increase in comparison to DMSO whereas GW1100 showed a decrease in comparison to DMSO, which was seen via the levels of uptake/intensity of the fluorescently conjugated Alexa 594-transferrin (Figure 3F, H). Thus, this confirmed that GPR40 in fact has an important role in the regulation of NMDAR endocytosis.

The molecular mechanism regarding GPR40 regulation of NMDAR-mediated excitatory synaptic transmission has yet to be uncovered, however, this study provided some preliminary support for GPR40 and NMDA receptor interaction. The surface expression of NMDARs was shown to have a critical role in NMDAR-mediated postsynaptic responses, and their mislocalization would therefore explain the possible pathological impact on epilepsy.

An interesting implication from this paper is the role that interactions between proteins had on the surface cell expression of NMDARs. More specifically, the reciprocal co immunoprecipitation, a technique that serves to precipitate a protein antigen out of a solution via the use of a specific protein binding antibody, showed that GPR40 directly interacts with NR2A and NR2B. Additionally, the activation of GPR40 led to decreased binding with NR2A/B, while the inhibition of GPR40 led to increased binding. This supports the idea that GPR40 is therefore involved in the regulation of both neuronal excitability and epileptic activity. The activation of GPR40 is thus thought to decrease epileptic seizures in animal models, as well as NMDAR-mediated postsynaptic transmission, which allowed for a new antiepileptic target to be established.[3] Overall, these results demonstrate that GPR40 activation decreases epileptic seizures through binding to NR2A/NR2B, inducing increased endocytosis of NMDA receptors, and therefore affecting NMDAR-mediated postsynaptic transmission and excitability.

4. Conclusion and Future Directions

Overall, more research is needed to understand the complex interaction between sleep and epilepsy. Sleep is a very important factor to consider in epilepsy patients and epileptic patients need to make sure they have a consistent sleep schedule.

In summary, Yang et al. found that increased GPR40 expression resulted in decreased binding to the NMDA receptor subunit NR2B which resulted in neuroprotective effects. One potential area of future research includes the downstream signaling interactions, as they can provide insight in the development of treatments.[3] In a previous study by Frasca et al., NR2B was studied by pharmacologically blocking NMDA receptors with Ifenprodil.[23] Their data suggests that the reduction of neurodegeneration during epileptogenesis was due to the block of excitotoxicity.[23] The findings of Frasca et al.[23] apply to the research discussed by Yang et al.[3], particularly in reference to the NR2B subunit. While the Yang study observed the binding of GPR40 to NR2B to be a method of NMDA receptor regulation, the Frasca study specifically examined the role of phosphorylation as a form of NR2B and NMDA receptor regulation. These two different approaches towards NMDA receptor regulation could influence future treatment development. The GPR40 and NMDA receptor interactions studied by Yang et al. showed an

increase in GPR40 expression. The phosphorylation of the NR2 subunit of NMDA receptors shown by Frasca et al. resulted in reduced cell death; perhaps an increase in GPR40 could induce the same effects.[3, 14] More research is required to determine if GPR40 is indeed a therapeutic target and can mediate the effects of epilepsy.

Yang et al. established a connection between GPR40 and NMDA receptors, which is a first and necessary step in studying this pathway further. On a gross anatomy scale, expression of GPR40 is localized to the hippocampus and cortex. Additionally, they found that GPR40 expression increased in epileptic brains compared to non-epileptic brains. These findings shed light on the effects of GPR40 on spine density and NMDA signaling amplitude.[3]

All of this further research can significantly contribute to the current understanding of epilepsy. This disease, characterized by the spontaneous recurrence of unprovoked seizures, significantly impacts the daily life of those afflicted with the condition.[1] Furthermore, advancements in the understanding of epilepsy can potentially shed light on the causes of other symptoms of the condition at a molecular level, such as confusion, anxiety, and deja vu.[1] Hopefully, the findings from Yang et al., along with future research on synaptic transmission in this field, can help push the scientific community one step closer to finding a treatment for this disorder.

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