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by

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ABSTRACT

THE ROLE OF α-AMINO-3-HYDROXY-5-METHYL-4-ISOXAZOLEPROPIONIC ACID (AMPA) RECEPTORS IN THE PATHOPHYSIOLOGY OF MIGRAINE PAIN

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Migraine is one of the most common neurological disorders, but remains poorly understood. It is characterized by severe throbbing, headache, and negatively impacts the quality of life of patients by causing attacks of debilitating pain. The mechanism by which migraines arise is not known, hindering the development of effective treatment options. Recent studies have demonstrated activation of glutamate receptors in the trigeminovascular system in the pathophysiology of migraines. In particular, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid (AMPA) ionotropic glutamate receptors have been implicated in the development of migraines. This study aims to investigate the role of AMPA receptors in the pathogenesis of migraine pain. Western blot analysis of brain tissue was utilized to quantify the changes in AMPA receptor subunit expression in mice that have been induced with chronic migraine pain. GluR2 expression was found to

be 3 times as high in mice induced with migraine pain than in the control group, supporting the involvement of AMPA receptor activation in the pathophysiology of migraine pain.

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CHAPTER 1

INTRODUCTION

Migraine is one of the most common neurological disorders, but remains poorly understood.[2] It is a severely disabling condition characterized by attacks of painful, unilateral, and pulsating headaches that can last up to 72 hours.[6] According to the World Health Organization, migraines are the second and third most prevalent disorders in the world and are ranked 7th on the most debilitating disorders out of 289 diseases surveyed. The overall prevalence of migraine is 18-25% of women and 6-8% of men in the United States with the most common types of migraine being tension type headaches (42%), migraine (11%), and chronic daily headache (3%).[6] Migraines are found to be a leading cause of work place absenteeism and among the most expensive neurological disorder, placing an economic burden on, not only the patient, but also on society.[6]

Despite the large prevalence of migraine pain, the mechanism by which migraines arise is not known, hindering the development of effective treatment options for migraine pain. Furthermore, the treatment options available to those suffering from this condition including nonsteroidal inflammatory drugs, opiates, and triptans are severely limited in efficacy, nonspecific, and carry the risk of side effects including overdose and liver and kidney damage.[6] Therefore, it is crucial to investigate the underlying mechanisms that contribute to migraine pain and explore targets for pharmacological intervention in order to develop effective therapies for the treatment of migraine pain.

There are two subtypes of migraines: migraine without aura (MO), which is characterized by nauseas, phonophobia, and photophobia; and migraine with aura (MA).[5] The activation of the trigeminovascular system (TGVS) has been implicated to contribute to the pain, and cortical spreading depression (CSD) is thought to underlie the symptoms of MA.[5]

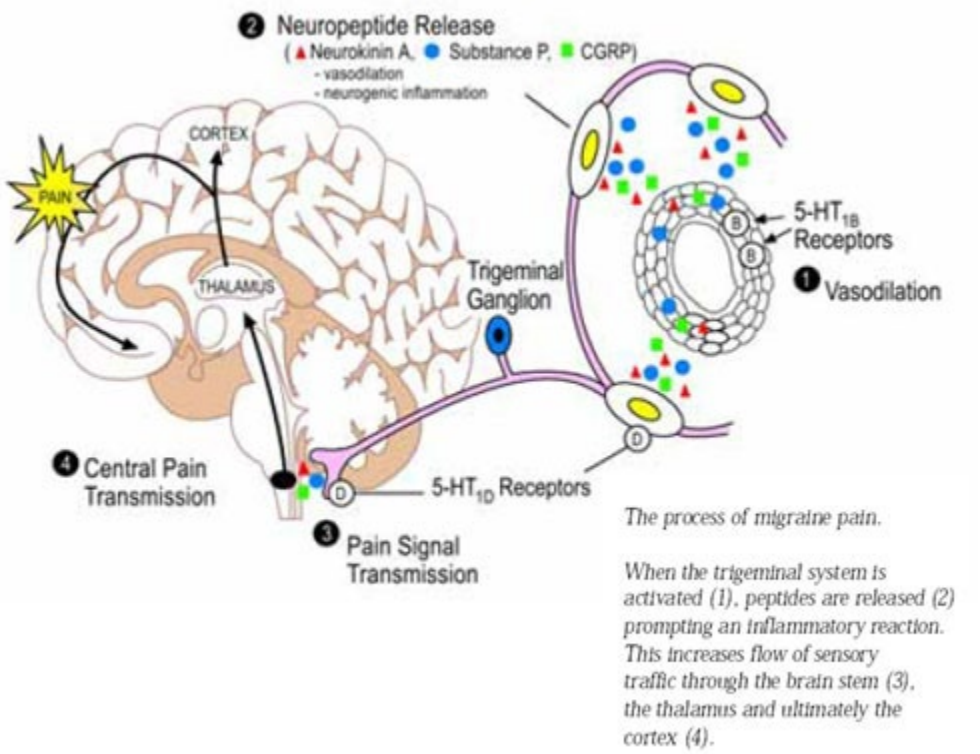

Figure 1.1: Activation of the Trigeminovascular System[8]

Pain sensitivity is caused by the activation of nociceptive afferent sensory fibers of the trigeminal nerve. This sensory information is carried from the meningeal trigeminovascular afferents to the trigeminal nucleus pars caudalis, thalamic nuclei, and periaqueductal grey area (PAG), which is known to modulate craniovascular pain.[5] Activation of these pathways results in the release of vasoactive neuropeptides including calcitonin gene related peptide (CGRP) which produces vasodilation of the meningeal

vessels and pro-inflammatory responses. Increased levels of circulating blood CGRP can be seen in patients suffering from migraine attacks. Sensitization of this pain network is thought to underlie serve migraines. The mechanism of TGVS activation is not known and what we aim to investigate in our study. [5]

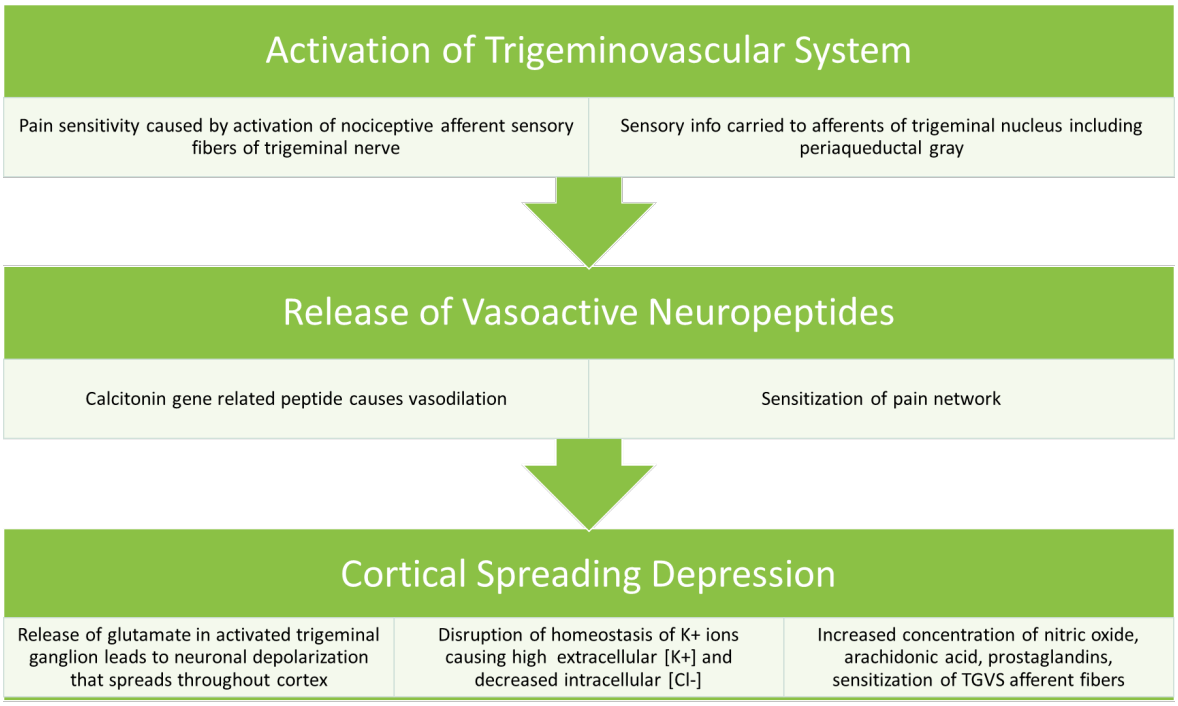

Figure 1.2: Proposed Mechanism of Migraine Pathophysiology

One neurobiological mechanisms of TGVS that has been hypothesized is CSD. CSD is postulated to result from the release of glutamate by NMDA and AMPA receptors present in the activated trigeminal ganglion.[5] This leads to a short-lasting wave of neuronal depolarization that spreads throughout the cortex. CSD produces disruption in the homeostasis of K+ ions causing a high concentration of extracellular K+ and a decreased concentration of intracellular Cl^-.[7] Additionally CSD increases the concentration of nitric

oxide, arachidonic acid, and prostaglandins leading to the sensitization of the TGVS afferent fibers. [5]

Furthermore, elevated glutamate levels have been found in the blood and cerebrospinal fluid of migraine patients. The failure of brainstem nuclei involved in the processing of central nociception is also hypothesized to be involved in the mechanism of migraine pain. [2] Therefore, glutamate receptors in this region are of great interest. It is crucial that the modulation of glutamate release by NMDAR, AMPAR, and kainite receptors be investigated in order to develop treatments for migraine pain.

Recent studies have demonstrated activation of glutamate receptors in the trigeminovascular system in the pathophysiology of migraines. [1,2] In particular, N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid (AMPA) ionotropic glutamate receptors have been implicated in the development of migraines. [2] Therefore, glutamate receptors may serve as a potential target for pharmacological intervention and the development of novel therapies for migraine pain. This study aims to investigate the neurological mechanism of glutamate in the pathogenesis of migraine and the efficacy of pharmacological inhibition of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the treatment of migraine pain.

METHODOLOGY

2.1 Conditioned Place Aversion

In previous migraine pain studies, animal models have utilized artificial stimulation of nociceptors in trigeminal afferents to induce chronic migraine pain. However, in this study, a novel animal model was established in which the nitroglycerin (NTG) induced chronic migraine when paired with conditioned place aversion. Acute migraine pain induced by formalin injections was paired with the naturally preferred chamber of c57bl/6 mice. After the conditioning period, animals were treated with the inflammogen NTG to induce a chronic pain state. Control animals were treated with saline.

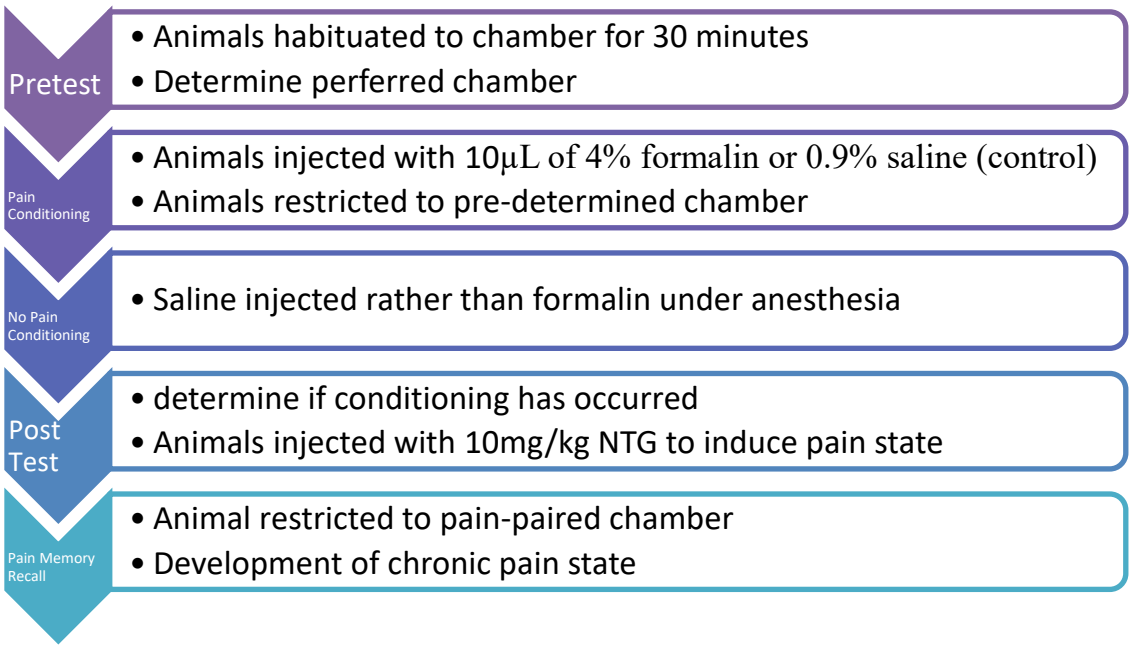

Figure 2.1: Conditioned Place Aversion Method

2.2 Von Frey Testing

Von Frey testing was utilized to evaluate orofacial cutaneous allodynia after NTG migraine pain was induced. The mice were habituated for 30 to 40 minutes. Following this, up-down testing occurred in which a filament was pressed firmly to the animal's forehead such that the filament bends in a sigmoidal curve. Pain behaviors including head shake, wiping the face with a single paw, or both head shake and wiping the face with a single paw were recorded. This is repeated with a lower force until no pain behaviors are observed. The experiment is repeated for 5-7 days until cutaneous baseline threshold is determined.

2.3 Western Blot Analysis

Brains were extracted from mice on day 44 of the experiment following CPA testing via perfusion. Brain tissue was homogenized in lysis buffer (RIPA; SIGMA #R0278) containing protease inhibitors (SIGMAFast Protease inhibitor tablets #S8820) and phosphatase inhibitors (Phosphatase inhibitor cocktail 3 #P0044). Samples were kept on ice at all times during lysis while proteolysis, dephosphorylation, and denaturation occurred. After homogenization was complete, the tissue samples were centrifuged in the homogenate at 10,000g for 10 minutes at 4°C in order to separate the protein (supernatant) from the cell debris (pallet). The supernatant was collected and used to make a 2x laemmli lysate (stored at -20°C or -80°C for long term use). Protein concentration was assessed using a bicinchoninic acid assay (BCA assay).

Following this, the proteins sample was heated to 95°C for 5 minutes to ensure protein denaturation. The sample was loaded onto an SDS polyacrylamide gel and run at 100V for 10 minutes until bands were resolved. The proteins were then transferred to a

PVDF membrane using wet transfer methods. The transfer occurred in transfer buffer composed of 100mL of 10x TG buffer (BIORAD #161-0771) with 200mL methanol and 700mL nanopure water. Transfer was carried out at 90V for 80-90 minutes on ice. The membrane was then removed from the transfer rig and blocked in 5% milk in PBST for 1 hour at room temperature with agitation. The sample was then stained with primary antibody specific for GluR1(Millipore AB1504) and GluR2 AMPA (Millipore AB 17687) receptor subunits in 5% milk in PST and sealed in a pouch overnight at 4°C. The blot was then rinsed in PBST three times for 10 minutes at room temperature and then placed in secondary antibody in 5% BSA and incubated for 1 hour at room temperature. The blot was rinsed in PBST three times for 10 minutes and stained with a Pico ECL Detection Kit to quantify changes in AMPA receptor and phosphorylated AMPA receptor protein expression. ImageLab software was used for quantification during western blot analysis.

CHAPTER 3

RESULTS

3.1 Increased Expression of GluR2 in Migraine Induced Mice

A greater expression of GluR2 AMPA receptor subunits was seen in mice treated with NTG in conjunction with CPA testing as well as in mice treated with 4% formalin only; and mice receiving no conditioning and 10mg/kg NTG. Mice treated with 4% formalin alone demonstrated the greatest level of GluR2 expression five times greater than the control groups (figure 3.1, figure 3.2). All mice induced with migraine pain, regardless of conditioning, expressed increased levels of GluR2 in comparison to the control group.

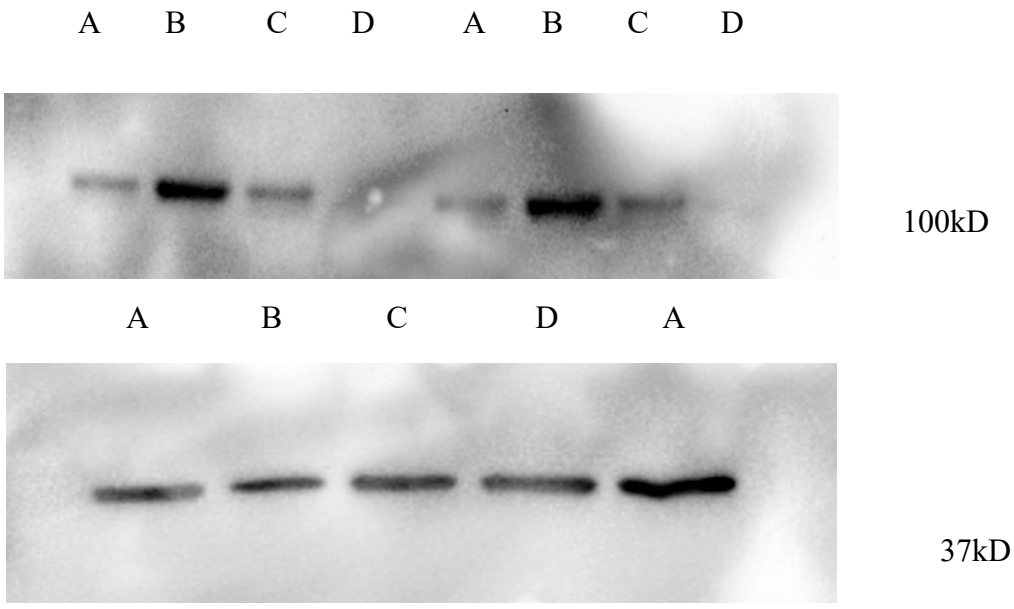

Figure 3.1: (Top) GluR2 Subunit Expression
A. NTG + CPA; B. CPA (4% Formalin); C. NTG;
D. Control (0.9% saline)
(Bottom) B-actin bands used for whole protein normalization;
A. NTG +CPA, B. CPA; C. NTG; D. Saline

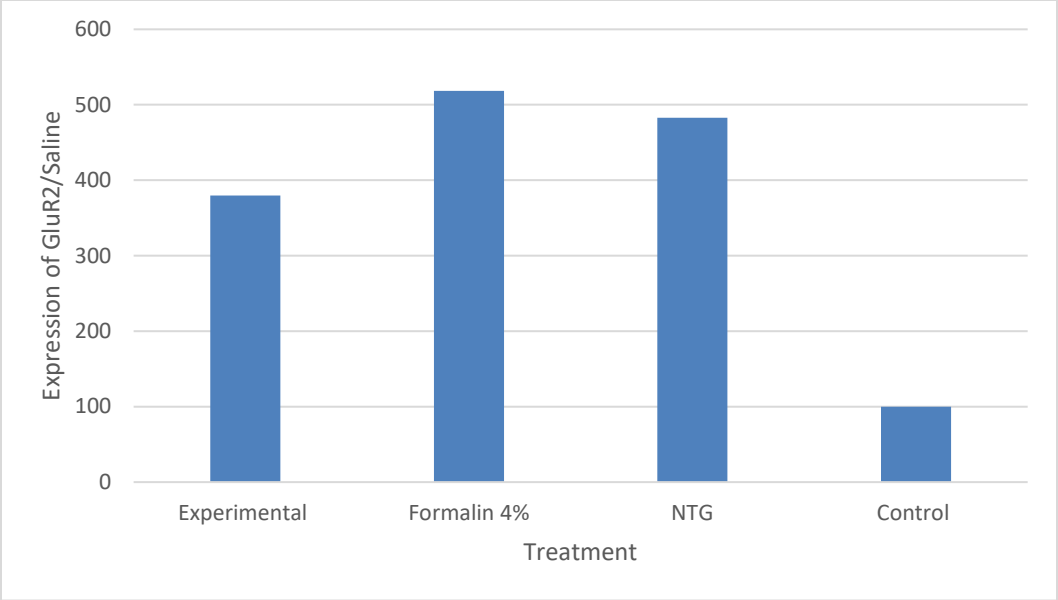

Figure 3.2: Expression of GluR2 Increased Among Treatment Groups. Experimental group (4% formalin + CPA +NTG) 3.8% increase in GluR2 expression in comparison to control; Formalin 4% (CPA only) 5.2% increase in GluR2 expression in comparison to control; NTG only 4.8% increase in expression in comparison to saline control group.

CHAPTER 4

DISCUSSION

Migraine pain is a severely disabling, recurring, multifactorial, and hereditary disease. The sensitization of trigeminal afferent fibers has been demonstrated in recent studies as a key component to migraine pain pathophysiology. However, the underlying molecular factors by which the activation of the trigeminovascular system occurs remains unknown. The dysfunction of glutamate receptors in this pathway has been strongly argued by numerous studies to lead to cortical spreading depression, a disruption of homeostasis in the brain, which causes generalized hyper-excitability.[2] Therefore, glutamate receptors may serve as a novel therapeutic target in the treatment of migraine pain.

This study was conducted to investigate the role of glutamate receptors, particularly AMPA receptors, in the pathophysiology of migraine pain. This was done by establishing a novel behavioral model for inducing chronic pain in mice. Conditioned place aversion was used in order to evoke pain with NTG, a known migraine trigger. Von Frey Hair testing was used to observe mechanical hypersensitivity. Molecular studies, including western blot analysis, were then employed to assess changes in AMPA receptor expression. Our preliminary findings have demonstrated an increase in the GluR2 subunit of AMPA receptors in mice throughout all treatment groups. However, due to the limitations of our study, our data is not sufficient to support evidence that AMPA receptors are involved in migraine pain This study must carried out more extensively with additional behavioral data

to confirm the induction of cortical spreading depression before any valid conclusions can be made.

Future studies will investigate the phosphorylation of AMPA receptor subunits to ascertain changes in intracellular signaling cascades, which lead to changes in expression of glutamate receptors. The involvement of AMPA receptor related cell signaling changes in the transition from acute to chronic migraine pain is also of great interest because those suffering from chronic migraine pain are at the greatest risk for negative side effects of conventional migraine therapies. Furthermore, migraine pain is more prevalent in females than in males.[1] Therefore, we aim to understand the molecular mechanisms underlying these sex differences. The efficacy of glutamate receptors as targets for pharmacological intervention and drug development is of great magnitude and must be further explored.

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BIOGRAPHICAL INFORMATION

Sameera graduated the University of Texas at Arlington with an Honors Bachelor of Science in Microbiology, as well as minors in chemistry and psychology. In her time as an Honors student, she served as an Honors Advocate, as well as the Honors College Council Newsletter Editor in 2015 and the Honors College Council Webmaster in 2016. Sameera will be attending the Texas College of Osteopathic Medicine following graduation in order to pursue her Doctor of Osteopathic Medicine. She plans on using her medical education to serve communities both in Arlington and abroad.