Potential regulatory mechanisms for NKCC1 and KCC2 that induce temporal lobe epilepsy

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Abstract. The specific cause for mesial temporal lobe epilepsy at a molecular level has remained unknown for decades. Much research has proposed possible mechanisms that would induce mesial temporal lobe epilepsy. In this article, we briefly summarize some of the present theories on the cause of this disease and then introduce our theory on NKCC1 upregulation in hippocampal pyramidal cells as a potential cause of epilepsy. We specifically discussed the WNK pathway that regulates NKCC1 and KCC2 as a potential cause of epilepsy and explored another possible mechanism that overrides WNK and would potentially induce mesial temporal lobe epilepsy. Eventually, we discussed future expectations on medication according to our theory.

Keywords: NKCC1, KCC2, Temporal Lobe Epilepsy, WNK pathway, regulatory mechanism.

1. Introduction

As a prevailing type of Temporal Lobe Epilepsy, Mesial Temporal Lobe Epilepsy happens mostly in the middle part of the temporal lobe, which involves the hippocampus and surrounding areas. Temporal Lobe epilepsy can be detected through MRI, where one can sometimes observe hippocampal sclerosis. It is also a widespread neuronal dysfunction in the central nervous system [1]. Additionally, several medications have already appeared to be efficient in treating mesial temporal lobe epilepsy, which includes Carbamazepine, Gabapentin, Lamotrigine, Topiramate, and Oxcarbazepine [2].

NKCC1, encoded by Slc12a2 and thought to be the cost of 30% of epilepsies, is a protein that is commonly found in neurons in the central nervous system, peripheral nervous system, and some glial cells and is responsible for the uptake of chloride ions from the extracellular matrix. NKCC1 predominates in the immature neuron, while KCC2 is kept in the minority due to some age-dependent factor, causing the chloride concentration higher within the cell, making the neuron excitatory. However, as the neuron matures, KCC2 upgrades and NKCC1 degrades, making the chloride concentration within and without cell alters, turning the neuron to be inhibitory [3].

Although not yet determined, the cause at a molecular level for mesial temporal lobe epilepsy had long been a prevailing topic, in this article, we focused on NKCC1 as a potential cause for this disease and try to set up a corresponding theoretical framework that justifies NKCC1 and its associated mechanism as a potential cause for mesial temporal lobe epilepsy, and will provide expectations for medicine accordingly.

2. Current Understanding Of The Potential Causes For Mesial Temporal Lobe Epilepsy

Taking up about 80% of all epilepsy, the cause of mesial temporal lobe epilepsy (MTLE) at a micro level remains unknown, it is often observed many macro or general causes would induce mesial temporal lobe epilepsy, which may include physical traumas in the brain area, genetic inheritances, and Neurocysticercosis according to a recently published paper [4]. Therefore, throughout the decades, researchers have hypothesized and testified several possible causes.

2.1. Reelin loss

There are varied explanations for the possible causes of MTLE, one of them that had been convincing in the decades is the theory about the loss of Reelin, a secreted neurodevelopmental glycoprotein, has long been thought to be associated with the formation of MTLE. In a paper in 2006[4], they proposed that loss of Reelin in the epileptic adult hippocampus induced aberrant integration of newborn Granule Cell Dispersions (GDC) [4]. Then later in a paper published in 2022, [5], they provided insight into the effect of Reelin, GDCs on mesial temporal lobe epilepsy, whereas they suspected that loss of Reelin affected GDCs, which is commonly found in epileptic brains [5]. This suspicion is then being supported by research in 2023 [6]. In this research, they found that loss of Reelin induced a decrease in its downstream target, disabled 1 (Dab1), which inhibits cofilin to avoid aberrant neuronal migration. Moreover, they found in the patient a significant overexpression of Cofilin, indicating the pathway they introduced to be quite plausible [6].

2.2. miRNA-induced epilepsy.

miRNA, as to Reelin, has been suspected for an extended period to be one of the causes of epilepsy. In a paper published in 2011 [7]. They first demonstrated a miRNA alternation after seizures [7]. Later, as more studies about miRNA's role in Temporal Lobe Epilepsy, the idea that miRNA affected some pathological responses in the brain area eventually led to epilepsy [8]. In a study in 2016 [9], they suggested that a decrease of generalized miR-146a-mediated leads to a decrease of complement factor H and is likely to induce temporal lobe epilepsy in the rat model [9]. Moreover, other studies suggested that miRNA is associated with neural function and plasticity that may also contribute as a factor for temporal lobe epilepsy [8]. For instance, in research in 2013 [10], the research group demonstrated that miRNA expression alternation induced functional changes in the dentate gyrus and thus had a large impact on causing temporal lobe epilepsy [10].

2.3. Blood-brain barrier (BBB) leakage induces temporal lobe epilepsy.

Unlike Reelin losses and miRNA dysregulation, Blood-Brain Barrier disruption is a rather new and minor area of consideration on what induced temporal lobe epilepsy. However, immune cells' transmigration or invasion into the brain parenchyma can also induce epilepsy [11]. This had been supported by a paper published in 2008 [12]. They demonstrated a pathogenetic link between leukocyte-vascular interaction, BBB damage, and the formation of seizures [12].

However, in a recently published paper in *2022*[11], the research group applied functional magnetic resonance imaging (fMRI) to 90 subjects with Temporal Lobe Epilepsy (TLE) and ran a statistical experiment to test the patients' functional connectivity in their brain areas. They demonstrated that the memory network of the TLE patients had experienced changes inside the mesial temporal lobe and frontal lobe when compared to the control group, and a higher level of disease burden is associated with weaker connectivity with the inter-mesial temporal lobe and intra-mesial temporal lobe, which support verbal and visual memory [11]. These new findings inspired a new possible mechanism for explaining the fundamental cause: the role of hippocampal pyramidal cells in causing mesial temporal lobe epilepsy.

3. Nkcc1 Upregulation In The Hippocampal Cells Induced Mesial Temporal Lobe Epilepsy

Hippocampal pyramidal cells are learned to be associated with spatial learning [13]. Thus, functional disability of hippocampal pyramidal cells would cause alternations in spatial learning and furthermore affects episodic memory. Moreover, hippocampal pyramidal cells contain NKCC1 and KCC2 proteins

[14], which are recently found to be key proteins in inducing epilepsy [15]. Therefore, we hypothesized such following mechanism: upregulation of NKCC1 associated with downregulation of KCC2 [16] induced intracellular chloride ions accumulation, thus, alternating the charge within the neurons turning it from inhibitory to excitatory [15], causing hyperexcitement, which damaged the original function of hippocampal pyramidal cells, causes epilepsies, and disrupts episodic memory. In some cases, a high level of intracellular chloride ions would become toxic for the neurons, therefore inducing more than 50% of cells in the hippocampus to go through apoptosis and inflammation, which on a macro scale would display as hippocampal sclerosis [17]. Additionally, this hypothesized mechanism follows the pattern of neural maturation. Since NKCC1 must experience a downregulation while KCC2 has to experience an upregulation as the neuron matures, any mutation or functional disability during this process of transforming would result in an outbalanced NKCC1 and KCC2 ratio, which makes the maturing stage of the neurons having the most threats for experiencing hyperexcitement. This provides a plausible explanation for why it is common for the mesial temporal lobe to display epilepsy since it is one of the areas that mature first in the brain [18].

3.1. WNK-SPAK/OSR1 function in regulating NKCC1 and KCC2 and as possible cause for epilepsy
Several recently published papers have extended our previous hypothesis and have adjusted our focus on the whole mechanism. In these two papers [19] [20], WNK had been demonstrated as both chloride concentration gated, and cell volume gated (Figure), whereas the cell is exposed to a hypertonic Cl environment, the cell would go through hyperosmotic.
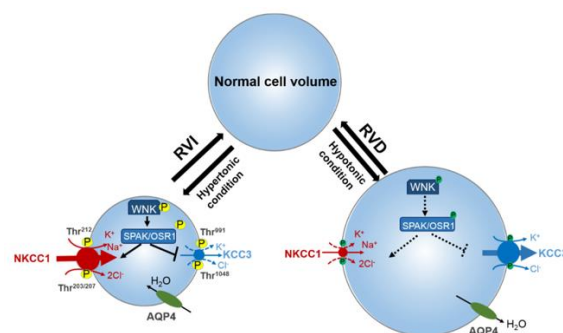

Figure 1. Roles of WNK, NKCC1, and KCC2 on regulating cell volume and intracellular ion concentration [20].

Thus, activating the WNK-SPAK/OSR1, and upregulating NKCC1 to bring in Cl to restore cell volume. Moreover, the WNK pathway had been found to regulate NKCC1 and KCC2 at the same time, and when WNK is activated, it upregulates NKCC1 as well as downregulates KCC2[19] [20]. And as research indicated that WNK has an autophosphorylation mechanism [21], it remained unclear how WNK function in immature neurons. According to existing researchers, in immature neurons where intracellular chloride is much higher than in mature neurons, the WNK pathway should be inhibited, and NKCC1 should be downregulated while KCC2 should be upregulated, but NKCC1 still prevails in immature neurons. There must present an unknown factor that regulates WNK and he co-transporters. Though some mechanisms had been found to furthermore regulate WNK, NKCC1, and KCC2 (Figure 2), more are yet to be discovered, since these factors, when malfunctioning or mutated, have the potential to disrupt the expression of WNK or directly disrupt the co-transporters, thus inducing hyperexcitement and causing epilepsy. Therefore, it is critical to identify as many as mechanisms to avoid many unknown causes of epilepsies as possible.
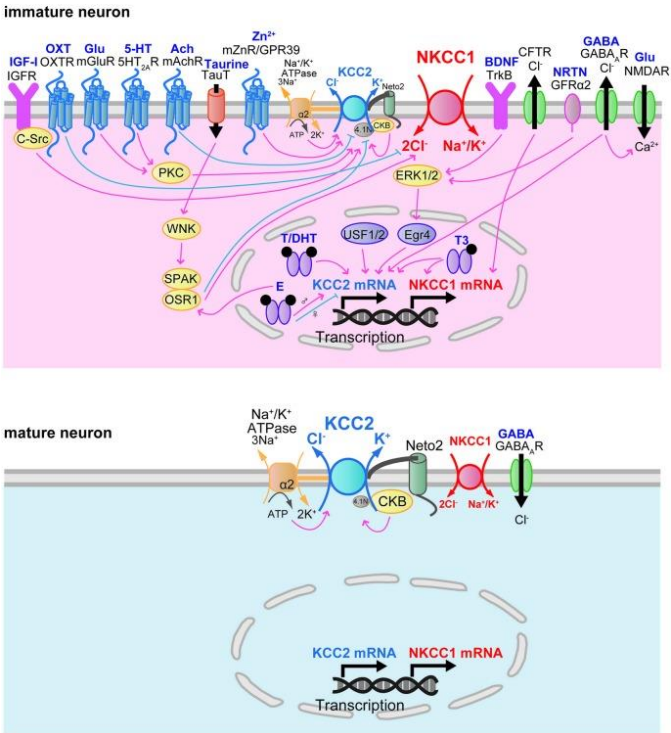
Figure 2. Currently known proteins for regulating WNK, NKCC1, and KCC2[18].

3.2. Possible mechanisms other than the WNK pathway in regulating NKCC1 and KCC2.
We further categorized the mechanisms that regulate WNK, NKCC1, and KCC2 into three categories: an unknown mechanism that regulates WNK, an unknown mechanism that regulates KCC2/NKCC1 along with WNK, different regulation patterns of WNK in immature and mature neurons (Figure3). And all three these categories do not stand against each other, they can be stand-alone, dependent on each other, and even coexist.
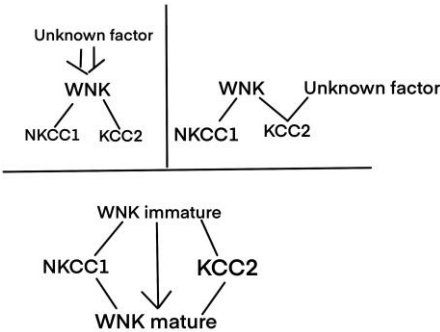

Figure 3. Three possible categories of mechanisms regulate WNK, NKCC1, and KCC2.

For the first categories, the unknown factor regulates WNK and furthermore regulates the co-transporters, has examples of Kelch-like 3(KLHL3) and Cullin-Ring ubiquitin 3 (CUL3) [22], which both would inhibit WNK kinase expression. KLHL3 BTB binds the C-terminus of L-WNK1 or WNK4, and Ring-box protein 1 of CUL3 binds to KLHL3 in the BTB domain, using enzymes that would degrade the two WNKs, and thus is inhibited [22]. Other than KLHLU3 and CUL3, Taurine happens to be another natural-occurring inhibitor or WNK family [18]. Another type of WNK regulator is SGK1, but SGK1 and WNK are in bilateral regulation (specifically for WNK1 and WNK4), whereas SGK1

phosphorylates WNK at S1169 and S1196[22], while the WNK family's N termini displayed effectiveness to strong activation of SGK1 [23].

In the second category that WNK and an unknown factor both regulate the co-transporters, a paper published in 2017 provided an example that when spinal alpha-7 nicotinic acetylcholine receptor (nAChR) is activated, it upregulates KCC2 in rats [24], whereas brain-derived neurotrophic factor/tyrosine receptor kinase B expressions are reduced by nAChR that leads to an upregulation of KCC2. Moreover, in a paper published in 2019, during Hypoxic-ischemic encephalopathy (HIE), hypoxia-inducible factor-1α (HIF-1α) upregulates NKCC1 and nuclear factor of activated T cells 5 (NFAT5) that down-regulates NKCC1 [25]. This mechanism serves another typical type of multiple factors regulation on the cotransporters.

The last category of the mechanism, the difference in functions in mature and immature central nervous systems, has a limited number of studies compared to the other two categories. However, there is still insight into this category. In a paper published in 2021, *WNKs are potassium-sensitive kinases* [26], proposed that potassium is a chloride-independent WNK inhibitor. Moreover, the paper demonstrated a potassium secretion increase when bathed in a hypotonic environment, following a WNK-, Fray-, NKCC- dependent manner [26]. This provides insight into a possible mechanism of WNK in an immature state of neurons, where we can hypothesize that WNK in immature neurons is more potassium-gated than chloride-gated.

4. Envisioned Treatment (Medication)

For mesial temporal lobe epilepsy induced by NKCC1 overexpression, we do not believe medications should be inhibiting NKCC1 itself, even though it's the direct cause of the disease. In most cases, the upregulation of NKCC1 often comes with the downregulation of KCC2 [19] [20], which makes the cell vulnerable to hypertonic environments and increases the percentage of apoptosis in such an environment. Thus, if a medication inhibits NKCC1 protein, it may release the symptom for a limited period, but it potentially decreases the cell's ability to respond to hypotonic environments as well, which would eventually proliferate the threat on cell apoptosis. Under such saying, we think the medication should be targeting the WNK pathway or other mechanism that regulates the co-transporters, wherein such means, we could restore the organism's ability for maintaining homeostasis, and thus self-cure epilepsy.

5. Conclusion

Through the analysis of the current understanding of mesial temporal lobe epilepsy, this article introduced several possible causes of mesial temporal lobe epilepsy and focused on one cause to analyze its current stage and propose future development. The article also includes envisioning medications for the specific cause of the disease previously described. The causes of epilepsy are diverse and largely varied, and the process of uncovering the causes at a molecular level could be time-taking and burdening. However, finding the cause of a disease at a molecular level means the discovery of potential targets, and potential biomarkers, which leads to a higher cure rate and early detection. Under such a saying, it has always been critical to seek the potential cause of every disease.

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