

Effect of sodium channels in Alzheimer's disease

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Abstract. Alzheimer disease (AD), depicted by the formation of amyloid β -protein plaque ($A\beta$), has been recognized for dementia and epilepsy. The pathological mechanism of AD is complicated and it remains unclear. Currently, most therapies are focusing on elimination of $A\beta$ plaque and preventing the breakdown of acetylcholine to prevent neuron cell death, however, most of these drugs fail to improve cognitive functions in AD patients. Therefore, the development of novel therapies for AD is in urgent need. Emerging evidences show that cellular sodium (Na^+) signals play a significant role in neuron physiology. $A\beta$ oligomer upregulates the expression of Nav1.6, leading to cognition loss, epilepsy, and malfunction of neuron development (shorter neurite length is observed during $A\beta$ -interfered neuron development), indicating that sodium channel may be a target for treating AD. This review summarizes current development of molecular mechanisms linking sodium dysregulation with AD pathologies and discusses potential therapies for AD by correcting Na^+ disruption.

Keywords: Sodium, Alzheimer's disease, Na^+ channels, therapy.

1. Introduction

Alzheimer's disease (AD) is one of the most well-known neurological disorders which impairs memorization, cognitive ability, and behavior. The hallmarks for identifying AD are formation of neurofibrillary tangles due to aggregation of tau, and amyloid plaque due to aggregation of $A\beta$ [1]. Mild cognitive symptoms occur at early stage, but people usually overlook the cognitive impairment and attribute it to aging. Therefore, many patients miss the best time for precaution and treatment. For Americans aged of 65 and older, AD ranks as the fifth biggest cause of mortality. If effective therapies are not identified, the current 5.8 million people with AD dementia could increase to 13.8 million by 2050 [2]. AD is the most frequent trigger of cognitive dysfunction for old people [3], and there is no panacea that can completely eradicate AD symptoms. Therefore, more studies are urgently needed to clarify the progression of AD. Neuron development and its function is a potential target for curing the cognitive deficit, since neuron function directly relate with cognitive ability. More and more evidences prove that under AD condition, neurons express hyperactivity [4], and epileptic symptoms are observed [5]. In order to understand the pathophysiology of neurons in AD, the role of sodium channel is inevitable. This review intends to summarize emerging roles of sodium in AD progression and offers an insight into manipulations of Nav1.1 and Nav1.6 in AD.

2. Roles of sodium channels in AD

Na^+ current plays a crucial role in neuronal function, the entry and exit of Na^+ across cell membrane is significant for controlling activation, propagation of action potentials, and back to resting potential [6]. Na^+ current plays a central role in neural signal transduction, including memory formation and pain feeling, which is mainly controlled by voltage gated sodium channels [7]. The dysfunction in sodium channel controlling the sodium current can lead to hypersynchrony and epilepsy [8]. Emerging research find that the whole sodium homeostasis will be deregulated with the overproduction and accumulation. Importantly, many deregulated Na^+ channels have been identified both in AD patients and experimental models.

2.1. Nav1.1

The Nav1.1 is made up with α and β subunits, and disulfide linkage of α and β subunits anchor the Nav1.1 on the cell membrane [9]. Kim et al. proved that $\beta 2$ is a target of BACE1 [10], and BACE1

also cleave APP to form β -CTF (C-terminal fragment), which contribute to accumulation of aggregates [11]. The regulation of $\beta 2$ has close relationship with expression of Nav1.1. In their experiment, they found that $\beta 2$ was firstly processed by BACE1 to form $\beta 2$ -CTF β , and $\beta 2$ -CTF β was then cut by γ secretase to produce $\beta 2$ -ICD (The ICD stand for intracellular domains). The $\beta 2$ could also be cleaved by α secretase to produce $\beta 2$ -CTF α , which was shorter than $\beta 2$ -CTF β , and $\beta 2$ -ICD was able to be produced from the reaction of $\beta 2$ -CTF α and γ secretase. The mRNA of Nav1.1 α was positively correlated with ADAM10, BACE1, or γ secretase expressions, showing that $\beta 2$ -ICD is the regulator of Nav1.1 α ; the more $\beta 2$ -ICD there is, the higher the expression of Nav1.1 α . Nevertheless, high expression of BACE1 was detected in AD mice samples, and it was found that BACE1 overexpression led to decrease in Nav1.1 expression on cell surface. Continuous research suggested that although Nav1.1 α is upregulated as the result of BACE1, large amount of Nav1.1 α accumulate inside cell, and only few Nav1.1 α can translocate to cell membrane. High BACE1 activity actually reduce Nav1.1 expression, probably due to insufficient $\beta 2$ that can bind with Nav1.1 α , since reduced sodium current is observed in $\beta 2$ knocked out mice.

2.2. Nav1.6

Presence of A β is a hallmark of AD, reports has shown that A β 1-42 can largely upregulate the excitability of Nav1.6, while it has no effect on Na⁺ current. Moreover, the increase in excitability of Nav1.6 is positively correlated with the concentration of A β added. A β also significantly increases the spiking frequency of Nav1.6 channel. The action potential threshold of neurons with A β added is more negative compared with control group [12]. All these results suggest that A β upregulate excitability of Nav1.6 in vitro. Meanwhile, Wang et al. tested the molecular kinetic of Nav1.6, and they found that compared with the control group, there was no statistically significant difference in A β treated group, suggesting that the hyperactivity of Nav1.6 and the elevated Na⁺ current might not be caused by amplification of sodium channel activity. Western blot results showed that Na⁺ channel expression was significantly increased after A β treatment, which was consistent with the mRNA expression, indicating that the target of A β might not be the channel itself, but the expression of Nav1.6 [13]. As a result, the Nav1.6 expression regulated by A β has a close relationship with AD.

Increased Nav1.6 was found to exacerbated cognitive defects [14]. It is known that AD suppresses long term potentiation, which results into synaptic plasticity deteriorates and leads to memory loss and learning deficit. Knockdown of Nav1.6 in AD mice with shRNA led to denser dendritic spine, upregulation of synaptophysin (a marker of postsynaptic density) and PSD95 (membrane-associated protein in postsynaptic density). Then, Yuan et al. specifically elucidated the relationship of Nav1.6 and BACE1. The APP/PS1 mice have high level of BACE1 and β -CTF, and knock down of Nav1.6 with shRNA lower BACE1 on both mRNA and protein aspect. β -CTF level reduced, but α -CTF and full-length APP expression doesn't change. Knockdown of Nav1.6 might attenuate A β formation by limiting BACE1 expression. Better learning ability was observed in Nav1.6 knocked down AD mice than other AD mice in Morris water maze test. The use of either Nav1.6 siRNA or TTX (non-selective sodium channel inhibitor) has no effect on wild type mice. However, both of them can reduce BACE1 mRNA/protein level with treatment of A β oligomer, showing that regulation of BACE1 by Nav1.6 is dependent on presence of A β . Yuan et al. also observed that dysfunction Nav1.6 reverse Na⁺ Ca²⁺ exchanger (NCX), result in influx of Ca²⁺ inside cell. Ca²⁺ accelerate A β formation and aggravate AD symptoms. Increased Ca²⁺ level stimulated activation of NFAT1, thus promoting BACE1 transcription. Upon activated, NFAT1 is dephosphorylated and moves into nucleus to perform its role as transcription factor. Experiment result proved that A β increased Ca²⁺ concentration in cell, but rescued by used of Nav1.6 shRNA, TTX, and KB-R7943 (Na⁺ Ca²⁺ exchanger inhibitor). Both Nav1.6 inhibition, Ca²⁺ chelating, and NCX inhibitor suppress NFAT1 level and hyperactivated BACE1 mRNA. By combining these results, A β activate NFAT1 via sodium current influx through Nav1.6 and NCX mediated calcium influx, and NFAT1 promote BACE1 transcription, leading to accumulation of β -CTF. In vivo result shown that, in AD mice, the p-NFAT1 level is lower and active NFAT1 is highly expressed. Knock down of Nav1.6 raise the inactive p-NFAT1 expression and lower

the active NFAT1 level. Nav1.6 knockdown attenuate Ca^{2+} influx induced by $\text{A}\beta$, reducing BACE1 upregulation, which may affect the progress of AD.

It has also been found that APPswe modification upregulated the $\text{A}\beta$ in cells, thus increasing sodium current [15]. Additionally, the delta E9 group (mutation that impairs gamma secretase function) has also found to be able to increase pattern in sodium current density, suggesting that the gamma secretase might have effect on the progress of AD. Another interesting thing is, the decay constant of both sodium current in M146V group (a mutation in transmembrane domain 2 in presenilin 1) and APPswe were faster compared with WT group, but there was no significant difference in rising constants. Another important phenomenon observed was that both PS1 mutant group and APPswe group showed shorter neurite length. The consequence of shorter neurite strength was the insufficient cellular electrical capacitance. Ghatak et al. keep tracking on the developmental pattern of AD neurons. The AD neurons at 2 weeks of culture showed elevated current densities of both sodium and potassium, higher electrical capacitance, and denser synapses compared with the wild type group at the same time, showing a premature morphology of AD neuron at early stage. As neurons getting mature, the electrical capacitance and neurite length gradually reduce, and sodium current density with raised action potential frequency shows the enhanced excitability in AD neurons, which may have effect on the progress of AD.

3. Correcting sodium dysregulation as a therapeutic approach in AD

There is increasing evidence to suggest that sodium current dysfunction actively participate in development and deterioration of cognition in AD, therefore, therapeutic approaches that correct Na^+ disruptions could treat neurodegeneration in AD.

Nav1.1 and Nav1.6 expression are inverse in AD, since Nav1.1 is downregulated and Nav1.6 is upregulated. Therefore, therapies can be addressed on enhancing Nav1.1, while suppressing Nav1.6 expression. Moreover, both Nav1.1 and Nav1.6 have close relationship with BACE1, and cleavage of hAPP by BACE1 lead to accumulation of β -CTF, which form $\text{A}\beta$ afterward. BACE1 might be a hub relating multiple aspects in AD pathology, which might be a future therapy direction.

Research suggested that the increased expression of Nav1.1 might counteract the epilepsy and hypersynchrony in AD. Aberrant interneuron network oscillations are observed in AD [16], and transplantation of interneuron progenitor might be an effective therapy. The researchers used J20 AD model, and the source of transplantation come from embryonic medial ganglionic eminence (MGE). MGE with overexpression of Nav1.1(MGNav1.1) and MGE with WT Nav1.1 expression (MGEWT) was bilaterally transplanted into J20 mice, and sham treated J20 and non-transgenic (NTG) were served as control. Compared with control, serious hyperactivity is observed in J20SHAM in open field and Y-maze, and the therapeutic effect of MGEWT on J20 is insignificant, however, the same symptom was not observed in J20MGE-Nav1.1. Additionally, transplantation of overexpressed Nav1.1 MGE cells can attenuate the epileptic symptoms in AD mice. Since AD patients usually suffer the hypersynchrony, therapeutic effect of Nav1.1 on epileptic symptom is tested. By monitoring the epileptic discharge (spikes/min), the researchers observed that the hyperactivity and high frequency rate are reduced in J20MGE-Nav1.1 compared with J20SHAM. Since the spike frequency is inversely correlated with exploratory behavior, reducing the high spike frequency is beneficial for suppressing $\text{A}\beta$ induced hypersynchrony. Moreover, in Morris water maze, J20MGE-Nav1.1, but not J20SHAM, favor the original location, indicating that transplantation of overexpression of Nav1.1 reduced cognitive deficit in $\text{A}\beta$ mice. The same experiments were operated in transplantation of MGE cells with reduced Nav1.1 (MGNav1.1 KI) in NTG and J20 mice. Results showed that NTGMGE-Nav1.1 KI showed longer escape distance compared with NTGMGE-Nav1.1. Meanwhile, compared with J20MGE Nav1.1 KI, J20MGE-Nav1.1 had better performance, suggesting that the reduced expression of Nav1.1 impaired learning ability. As a result, increasing Nav1.1 could be a new treatment for AD.

Anisomycin was found able to suppress the hyperactivation of Nav1.6 by activating p38 kinase [9], meanwhile, p38 kinase phosphorylate the serine-proline dipeptide on L1 of Nav1.6, and Na channel

function is inhibited [17]. Activation of p38 kinase might be a novel therapy to rescue hyperactivation of Nav1.6 in AD.

Moreover, research conducted by Kobelt et al. shed light on procedure that promote neurite growth [18], which might be used to counteract the malformation development of neurite in AD which mentioned earlier [11]. Kobelt et al. suggest that the use of electrical field stimulation with cytokines such as, epidermal growth factor (EGF), fibroblast growth factor (FGF), and interferon gamma (IFN- γ) can facilitate neurite growth. The length of neurite increased with those cytokines added and supply of 0.53V/m and 1.83V/m DC electric field stimulation comparing with control group [18]. Ghatak et al. suggested that the shortened neurite led to reduced electrical capacity during development of AD neurons [11]. Elongation of neurite length with cytokines and DC electrical stimulation might ameliorate the neurite loss, since bioelectricity and proper cytokines transduction are necessary in healthy neuron development. Ghatak et al. prove that inhibition of β secretase or γ secretase can reduce the hyperexcitability in AD neurons [11], but it is still unclear that the curative effect should attribute to insufficient A β formed since its producer β secretase or γ secretase is inhibited, higher Nav1.1 expression, or both of these factors contribute to therapy. Mutation that impairs gamma secretase function shows similar increase pattern in sodium current density, suggesting that the gamma secretase might be a target for future therapy [19].

4. Conclusions

Nowadays, research groups all over the world made tremendous progress on understanding the cause and mechanism of AD, but the perplexing aetiological agents make development of effective therapy difficult, and there is no single effective treatment that can cure AD thoroughly. Apart from the complexing mechanism of AD development itself, another factor dampening the drug development process is lack of experiment data *in vivo*. Large amount of creative therapeutic plans is provided in mouse model or *in vitro* model, and the understanding of AD in human will be more elaborated if more researches can be conducted *in vitro*. Sodium channels are extensively researched in neurodegenerative diseases, and attentions are addressed on the contribution of epileptic behaviors by dysregulation of Na⁺ current. Relationships between A β and expression of Na channels also studied in several aspects, including effect of A β oligomers on development of neurite, expression of Na channels, and regulations of BACE1, etc. Not only should the research of Na ion channels be expanded in the future, but so should the interaction of Na ion channels with other ion channels, such as Ca²⁺ channel. Research on BACE1 should also be accentuated, since BACE1 regulate expression of Nav1.1, and at mean time affected by expression of Nav1.6. Moreover, BACE1 can interact with Na⁺-Ca²⁺ exchanger, affecting Ca²⁺ influx, and BACE1 cut hAPP (precursor of A β) to form β -CTF. BACE1 might be the node in AD pathogenesis network.

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