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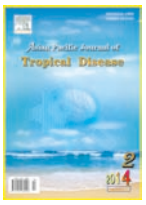

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Calcium regulation and Alzheimer's disease

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ABSTRACT

Activation of the neuron induces transient fluctuations in $[Ca^{2+}]i$. This transient rise in $[Ca^{2+}]i$ is dependent on calcium entry via calcium channels and release of calcium from intracellular stores, finally resulting in increase in calcium levels, which activates calcium regulatory proteins to restore the resting calcium levels by binding to the calcium-binding proteins, sequestration into the endoplasmic reticulum and the mitochondria, and finally extrusion of calcium spike potential from the cell by adenosine triphosphate-driven Ca^{2+} pumps and the Na^{+}/Ca^{2+} exchanger. Improper regulation of calcium signaling, sequentially, likely contributes to synaptic dysfunction and excitotoxic and/or apoptotic death of the vulnerable neuronal populations. The cognitive decline associated with normal aging is not only due to neuronal loss, but is fairly the result of synaptic connectivity. Many evidences support that Ca^{2+} dyshomeostasis is implicated in normal brain aging. Thus the chief factor associated with Alzheimer's disease was found to be increase in the levels of free intracellular calcium, demonstrating that the excessive levels might lead to cell death, which provides a key target for the calcium channel blockers might be used as the neuroprotective agents in Alzheimer's disease.

1. Introduction

The human body starts degenerating with increasing age and the most common neurodegenerative disorder that affects the elderly population worldwide is Alzheimer's disease. It is the most common form of dementia in the human population affecting primarily older age groups (60+) though its prevalence is believed to be increasing due to changing lifestyle habits and exposure to environmentally hazardous chemicals[1]. In 2010, there were more than 30 million people suffering from Alzheimer's disease (AD) but it is anticipated that by 2015 over 35 million people worldwide will be suffering from this disease[2].

Alzheimer's disease is an irreversible progressive devastating neurodegenerative disorder characterized by various intracellular and extracellular biochemical changes that result in the neuronal death. Elevation of beta-amyloid protein, indicated by the presence of extracellular plaques and formation of intracellular neurofibrillary tangles formed due to hyperphosphorylation of tau protein are the major pathological hallmarks of AD.

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Calcium plays a pivotal role in neuronal physiology by long term and short term regulation. Short term regulation of activity is through neurotransmitter release from the presynaptic nerve terminals while the long term regulation is through long term potentiation (LTP) and long term depression forms of synaptic plasticity in the formation of memory[3,4]. The neurons involving learning and memory are the most vulnerable areas for degeneration, the memory processing pathways like long term potentiation, long term depression forms of synaptic plasticity are affected mainly due to excessive release of calcium which leads to death of the cells by excitotoxicity. Calcium regulation is known to be the main key involved in the pathophysiology of Alzheimer's disease so, better therapy may be obtained by targeting calcium entry into the neurons.

2. Calcium regulation in healthy brain

The normal brain functions are evident at synapses by the release of neurotransmitters leading to a number of biochemical events in the post synaptic neurons. The most prominent among these events is rapid and transient increase in calcium levels.

Maintenance of calcium dynamics according to time and

magnitude is critical for neuronal activity. Neuronal cytosolic calcium under resting conditions is maintained at a steep gradient between low intracellular free calcium and high extracellular calcium. Intracellular free calcium concentration $[Ca^{+2}]i$ in neurons is maintained at 10^{-7} mol/L, while the extracellular free calcium concentration at 1.2 mmol/L[5]. Biological responses occur in the range of 100-500 nmol/L and pathological responses are elicited at intracellular calcium concentrations exceeding 1 μmol/L.

Normally calcium regulation is maintained by calcium channels, calcium release from internal stores, calcium binding proteins, calcium sequestration by intracellular organelles and energy-dependent calcium extrusion pumps[6]. Any disturbance in these molecular mechanisms leads to elicitation of pathological response.

Efflux and influx of calcium via the plasma membrane is mediated by the calcium channels. The calcium influx is mediated mainly by the voltage gated calcium channels and receptor operated calcium channels. Voltage-gated calcium (Ca^{2+}) channels are the key transducers of membrane potential[7]. They allow the passage of ions passively down the electrochemical gradient and regulate fast and slow components of calcium movement in neuronal endings, the Ca_v1 subfamily (Ca_v 1.1, Ca_v 1.2, Ca_v 1.3, and Ca_v 1.4) of L-type of Ca channels are mainly implicated in the hippocampal memory formation.

L-glutamate is one of the primary excitatory neurotransmitter in the central nervous system. The glutamate acts on ionotropic receptors which mediate direct Ca^{2+} entry into the cell through kainate, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and N-methyl-D-aspartate (NMDA) receptors. Of these NMDA receptors are prominent conductors of Na^+ and Ca^{2+} ions and function in excitatory post synaptic currents[8]. Therefore, excitatory post synaptic potential produced by NMDA receptor activation highly increases the levels of intracellular calcium. Primarily the L-type of calcium channels are the core channels involved in glutaminergic transmission that plays an important role in synaptic plasticity of memory formation[9].

In order to maintain the calcium homeostasis, the accumulated intracellular calcium has to be removed from the cell and the presence of a large concentration gradient across the plasma membrane makes Ca^{2+} efflux difficult as the passage of ions is against the concentration gradient. So the Ca^{2+} efflux requires energy dependent mechanisms[10], and the two major pathways responsible for calcium extrusion are the major transport systems capable of swiftly extruding significant amounts of Ca^{2+} from the cell, across the plasma membrane to extracellular space and maintains the levels of cytosolic calcium. They are sodium-calcium exchanger of mitochondria and plasma membrane Ca^{2+} adenosine triphosphatase (ATPase) pump.

Calcium binding proteins (CaBPs) are the neuroprotective proteins that bind to free calcium ions within the cell that helps to regulate intracellular calcium[11]. A moderate rise in intracellular calcium is maintained at an equilibrium state by rapid calcium buffering by the calcium binding proteins in the cytoplasm[12]. Thus, neurons protect themselves from the toxic effects of increased intracellular calcium spikes by buffering with CaBPs.

The main important function of the CaBPs is the mediation of calcium signals. They regulate the activity of downstream targets. The second function is direct Ca^{2+}-dependent enzymatic activity. The third function is buffering of intracellular Ca^{2+} and modulates levels of intracellular Ca^{2+}[13].

In addition, intracellular calcium levels are regulated by calcium stores present in the intracellular organelles, like endoplasmic reticulum (ER)[14], mitochondria[15], Golgi complex, and lysosomes[16].

These stores act as buffers to maintain resting intracellular Ca^{2+} levels[17]. ER is partially filled with calcium at rest, and the influx leads to increased concentration of calcium stored within the ER. This rise in concentration initiates the sequestration of Ca^{2+} through sarco endoplasmic reticulum Ca^{2+} ATPase (SERCA) pumps, and releases calcium in response to the activation of inositol 1,4,5-tris-phosphate receptor and ryanodine receptor[18]. Similarly mitochondria appear to contain little calcium at rest, and upon stimulation intracellular calcium rises, and this rise in calcium acts as a biosignal. Thus, the calcium buffering takes place through mitochondrial uptake, while nicotinic acid adenine dinucleotide phosphate (NAADP) mobilizes calcium from lysosome-related acidic compartments and this NAADP mediated calcium release increases the neurotransmitter release[19].

3. Calcium regulation in aging brain

Aging in humans is the greatest risk factor accompanied by conventional structural, physiological and neurochemical changes in the brain with variable degrees of cognitive decline[20]. All the aging brains show a gradual decrease in the ability to handle and retain both the existing and new memory, which is due to the impairment of neuronal activity in different areas of the brain[21].

Many theories have been proposed to explain the process of aging but none of them are satisfactory, one of the most common theory proposed was the free radical theory developed by Dr. Denham Harman, which states that the accumulation of oxidative stress was the key reason for aging[22]. Even though the brain accounts for less than 2% of the body weight it consumes 20% of the basal oxygen up take. Increased oxygen consumption is linked to leakage of electrons along the respiratory chain with subsequent formation of the free radicals[23]. The presence of large amounts of polyunsaturated fatty acids in the neuronal membrane was the other reason for accumulation of oxidative stress in the brain[24]. These polyunsaturated fatty acids undergo lipid peroxidation which results in the formation of the free radicals like malondialdehyde (MDA) and 4-hydroxy-2,3-nonenal which covalently modifies the proteins on the amino acids cysteine, lysine and histidine[25], which results in the formation of abnormal proteins.

4-Hydroxy-2,3-nonenal in turn leads to delayed elevation of intracellular calcium and this excess of calcium leads to apoptotic death of the neurons.

Aging alters the core components of the calcium regulation like calcium channels, calcium binding proteins, calcium sequestration by intracellular organelles and calcium extrusion pumps.

Calcium channels are involved in diverse physiological and pathophysiological functions in neurons. Evidences suggest that the neuronal voltage gated calcium channels in central nervous system alters with aging. Earlier studies found that long-term and short-term potentiation forms of synaptic plasticity which are highly dependent on calcium influx were altered in the hippocampus of aged rats[26]. In addition, a series of pharmacological studies have shown that the L-type Ca^{2+} current appears to be the primary high voltage activated component that is increased in aging CA1 neurons[27].

It was reported that the Ca^{2+} dependent after-hyperpolarization (AHP), the Ca^{2+} action potential and voltage-activated Ca^{2+} currents increased in aged principal cells of hippocampal CA1 neurons. Multiple types of Ca^{2+} channels appear to be affected by aging[28]. As with the Ca dependent slow AHP, calcium action potentials were also longer and larger in the aged neurons[29], providing strong evidence that the aging change in the AHP was due to changes in the properties of Ca^{2+} channels rather than in the K^{+} channels that mediate the calcium-activated AHP.

Normal brain aging is associated with a shift in the LTP mechanism of NMDA dependent to voltage dependent calcium channels[30]. In addition, aging increases the calcium induced calcium release from intracellular stores[31], and reduces the contribution of mitochondria to Ca^{2+} clearing processes[3]. These processes, together with the increased Ca^{2+} entry through the voltage gated calcium channels, will lead to increased Ca^{2+} loads and consequent increase in slow AHP, which may well contribute to the reduced NMDA dependent LTP, since the slow AHP decreases the depolarization required for NMDA receptor activation[32].

Extrusion of intracellular Ca^{2+} diminishes with age, as some key extrusion systems in Ca^{2+} regulation like Ca^{2+}-ATPases have been found to be reduced with age but the molecular mechanism responsible for elevated intracellular calcium levels remains unknown[33]. It was also found that Ca^{2+} extrusion by membrane pumps/proteins reduced in aging animals[34]. However, later Michaelis *et al.*, found evidence for a role of oxidative changes in the impairment of Ca^{2+}-ATPase function with aging[4].

A change in calcium regulation also occurs in response to hormones and the neurotransmitters which result in increase in the mitochondrial matrix concentration[35]. Calcium ions rush into the cristae resulting in the accumulation of calcium in the mitochondria, the accumulated calcium in mitochondria is subsequently released via Na^{+}/Ca^{2+} exchanger[36]. This excessive calcium taken up into the mitochondria increases the production of reactive oxygen species, ATP synthesis inhibition, induces the opening of the mitochondrial permeability transition pore, and the release of cytochrome-c and apoptosis-inducing factor triggering initiation of apoptosis, from the mitochondrial inter membrane space into the cytoplasm[37].

Most of the calcium entering into the cell is rapidly buffered by CaBPs[38], but most of these proteins were found to be effected in aging. Studies were reported on the declination of neuronal parvalbumin, calbindin in aged animals[11]. Aging also affects another important intracellular Ca^{2+} buffer, calmodulin. Michaelis *et al.*, have demonstrated that calmodulin isolated from aged rat brain is much less effective in stimulating plasma membrane Ca^{2+} ATPase activity than that isolated from the young rats. This effect may be due loss of conformational stability of calmodulin due to oxidative modification of several key methionine residues[34]. Finally, the fast Ca^{2+} buffering is dissociated in basal forebrain neurons of aged rats when compared to neurons of young rats[39].

Mitochondrial calcium sequestration declines with aging and this may be attributed to overall reduction in intracellular calcium buffering[40], and studies on synaptosomes isolated from aged neurons shows the mitochondrial reduction in calcium sequestration with age. This reduced sequestration of the divalent cation with age appears mostly due to decrease in the affinity of the mitochondrial Ca^{2+} uniporter[41]. Calcium sequestration in ER of aged neurons also found to be weakened due to diminished activity of the SERCAs[42].

4. Calcium regulation in Alzheimer's disease

The pathogenesis of Alzheimer's disease is complex; it involves much molecular, cellular and physiological pathology. It has been reported that cytotoxicity to neurons was induced by hyperphosphorylated tau found in neurofibrillary tangles. Evidences indicate that the state of tau phosphorylation is tightly regulated and seems to be calcium dependent. Cyclin-dependent kinase 5 and glycogen synthase kinase-3b are involved in AD-like phosphorylation of tau[43]. A transient increase in intracellular calcium concentration was previously demonstrated to induce a glycogen synthase kinase-3b-mediated phosphorylation of tau[44]. It was also reported that net dephosphorylation or phosphorylation of tau is dependent upon the rate and/or extent of calcium influx[45].

This neurodegeneration induced by amyloid-β (Aβ) or tau was mediated by the changes in calcium homeostasis.

Aβ disrupts calcium homeostasis probably by two major mechanisms. They include the formation of calcium conducting pores and formation of reactive oxygen species (ROS) through accumulation of oxidative stress[35].

It is also well documented that Aβ can form a novel calcium conducting pore in lipid bilayers providing a route through which uncontrolled calcium entry may perturb the tightly regulated intracellular calcium environment. Aβ peptides of various lengths, including $Aβ_{25-35}$, $Aβ_{1-40}$, and $Aβ_{1-42}$, have been observed to elicit cation selective currents when reconstituted into lipid bilayers[46]. When reconstituted

in liposome prepared with mixture of natural phospholipids such as phosphatidyl choline and phosphatidyl serine, $A\beta_{1-42}$ and $A\beta_{1-40}$ allow significant uptake of calcium ions in a dose-dependent manner[47]. In addition to the ability of Aβ to form these calcium conducting pores/channels in the plasma membrane, extracellular application of Aβ leads to drastic increase in IP3-mediated calcium release[48].

Aβ can disrupt Ca^{2+} homeostasis by its ability to form ROS which induces membrane lipid peroxidation that leads to alteration in membrane properties and affects the channels, membrane transporters, which results in elevation of intracellular calcium[49]. The Aβ oligomers generate hydrogen peroxide and hydroxyl radicals by interaction with Fe^{2+} and Cu^{2+}[50], and during this aggregation process at cell membrane lipid peroxidation is initiated which results in the production of toxic lipid aldehydes like 4-hydroxynonenal, a neurotoxin which impairs the functioning of the ion motive ATPases, glucose and glutamate transporters, guanosine triphosphate-binding proteins, ion channels by covalent modification of cysteine, lysine and histidine residues on proteins[49].

Aβ plays a vital role in disrupting intracellular calcium homeostasis. For example a number of studies have shown that Aβ can alter the activity of number calcium conducting ion channels such as voltage-gated calcium channels[51], NMDA receptors and nicotinic receptors[52,53].

The underlying molecular mechanisms responsible for this enhancement in calcium release from the ER remains to be determined, but it may be due to the combination of an enhanced intracellular calcium store and/or a failure by the cell, caused by the pathogenic actions of Aβ to regulate calcium.

Aging and Aβ constantly promote Ca^{2+} influx into neurons through L-type calcium channels. Soluble intraneuronal Aβ oligomers, soluble and insoluble Aβ fibrils can increase intracellular Ca^{2+}, impairs the neuronal function, and adversely affects synaptic functions in AD[54]. Increase in Ca^{2+} occurs through the over activation of L-type calcium channels[52]. The uncontrolled rise in Ca^{2+} can trigger the over expression of plasma membrane L-type calcium channels subtype Ca_v 1.2 in the hippocampus of AD brains and further exacerbate the pathogenic Ca^{2+} influx. Ca_v1.2 expression is essential for LTP (independent of NMDA dependent LTP), synaptic plasticity, and spatial memory of the hippocampus[55].

In addition to the effects that Aβ has on L-type, Aβ has also been shown to enhance Ca^{2+} current through the N- and L- type voltage gated calcium channels in synaptosomes and the N- and P- type channels in cortical neurons[56].

As reported that the oxidative changes in impairment of Ca^{2+}-ATPase functions with aging, the pathogenic mechanism through which Aβ has been shown to mediate its effects is via the production of ROS. During the process of Aβ aggregation, hydrogen peroxide and hydroxyl radicals are generated that can result in lipid peroxidation with a subsequent disruption in the activity of ion motive ATPases that are intimately involved in regulating intracellular calcium elevations (such as the calcium-ATPase)[57]. These neurotoxic actions of Aβ are not just limited to plasma membrane structures but also to other cellular components.

In addition to the declination in normal aging, calcium binding proteins like calmodulin (CaM), calbindin also change with Alzheimer's disease. It was found that CaM extracted from the Alzheimer's neocortex was less functionally active than CaM extracted from non-Alzheimer's dementia neocortex. Similarly calbindin reduction with age is further promoted in the Alzheimer's disease. Mclachlan *et al*., found a significant reduction in calbindin in neocortical areas of Alzheimer's subjects. CaM was reduced by 30% in the area of CA2 in Alzheimer's brain while calbindin reduced by 35% in CA1 and CA2 areas of Alzheimer's hippocampus[58].

5. Conclusion

Is blocking calcium entry into neurons is Neuroprotective? From the evidences and reports it was clear that the excessive levels of intracellular calcium is lethal to the cell, it is probably acting as a neurotoxin disturbing the entire calcium homeostasis. Many drugs targeting calcium signaling pathways have been demonstrated, few have been successful in clinical trials such as memantine[59], dimebon[60], nimodipine[61], and all these target plasma membrane calcium channels rather than intracellular signaling pathways that appear to be the main locus of calcium dysregulation in Alzheimer's disease. So, the logical therapeutic approach is to maintain Ca^{2+} homeostasis and prevent calcium from entering the cell or triggering its downstream neurodegenerating targets. Some of the avenues for maintaining calcium homeostasis include blocking calcium channels[62], glutamate receptors[63], intracellular calcium release[64], and the use of antioxidants[65].

As it was earlier reported that blockade of T-type voltage gated Ca^{2+} channels offers neuroprotection with channel blockers like Isradipine[66], it inhibited the calcium conductance of L-type Ca_v 1.2 channels, due to the increased expression of Ca_v 1.2 receptors in AD[67]. These calcium channel blockers decreased the availability of the Ca^{2+} at the neuronal sites which might be neuroprotective. More specific targets that target intracellular Ca^{2+} release, SERCA pumps, Aβ formed calcium channels, mitochondrial calcium system, calcium regulatory proteins may provide new therapeutic opportunities for future.

Blockade of glutamate receptors offers neuroprotection as the glutamate induced cell death can be prevented, upon prolonged exposure follows a rapid influx of Na^+ and Cl^- with cell swelling, and with massive influx of Ca^{2+}, activation of Ca^{2+} mediated cascades leading to neuronal injury and cell death[61], here also the role of calcium appears to be crucial, one such drug that is being used is memantine.

It was also reported that oxidative damage is the earliest event that could be detected in brain prior to the formation of plaques and tangles, and the ROS also increases the calcium stores by inhibiting the mitochondrial enzyme so, the use of the antioxidants which prevents the conversion of

Aβ monomers to Aβ oligomers, can offer better protection as the oxidative stress increases the formation of oligomers and finally neurofibrillary tangles[68].

Conflict of interest statement

We declare that we have no conflict of interest.

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