Calcium: A proven target in the war on Alzheimer's disease

Abstract

Alzheimer's disease is practically a household word these days, as the number of individuals diagnosed with this debilitating condition continues to skyrocket. While effective treatments are in the developmental stages, many promising areas of scientific research are demonstrating that several different types of cellular pathways are involved in the death and destruction of critical brain cells. The role of a single yet powerful molecule, **calcium**, is now at the forefront of such research, as diverging lines of evidence point to the role of calcium in healthy and unhealthy brain cells. Numerous signaling pathways that work to keep brain cells functioning at their optimal level involve calcium. Researchers and clinicians agree that a better understanding of the effect that calcium has on brain cells may hold the key to eventually halting the progression of Alzheimer's disease and dementia. One class of proteins in particular, calcium-binding proteins, has the powerful ability to influence the activity of calcium in brain cells. One such calcium-binding protein, **apoaequorin**, has been utilized by researchers for decades but more recently has been under scrutiny as a potential therapeutic in the control of calcium levels in the brain. This protein, like others in its class, represents a new direction in the race to discover more effective Alzheimer's disease prevention and treatment modalities.

The reality of Alzheimer's disease and dementia in today's world

Nearly every 70 seconds, someone new in the United States develops Alzheimer's disease (AD). By the year 2050 this rate is expected to accelerate to every 33 seconds (2011 Alzheimer's Disease Facts and Figures).

Now the sixth leading cause of all deaths in the U.S., Alzheimer's disease continues to rise dramatically. This phenomenon is unlike many other highly fatal illnesses such as heart disease, stroke and prostate cancer. Preliminary data reported in the 2011 edition of "Alzheimer's Disease Facts and Figures," an annual report released by the Alzheimer's Association, indicate that between the years 2000 and 2008 stroke deaths decreased by 20 percent and heart disease deaths decreased by 13 percent. Deaths caused by AD, on the other hand, increased by 66 percent in that eight-year period.

The effect that Alzheimer's disease has on those living with the disease is equally stunning. Classified as one type of dementia in the larger family of dementias, AD is the most common. Alzheimer's disease affects an estimated 5.4 million Americans and 18 million people worldwide. Future projections paint

an even grimmer picture. In 2050, the incidence of Alzheimer's is expected to approach nearly a million per year, and the total estimated prevalence of individuals affected in the U.S. will reach 11-16 million.

Aside from the emotional toll that AD takes on affected individuals and their families, the financial burden represents another overwhelming challenge. Total expenditures in 2011 for health care, long-term care and hospice services for patients aged 65 years and older with AD and other dementias are expected to reach $183 billion; this estimate does not include the contributions of unpaid caregivers. In the year 2010 alone, nearly 15 million family members and other unpaid caregivers provided an estimated 17 billion hours of care to individuals with Alzheimer's and other dementias. This contribution was calculated to be valued at more than $202 billion (2011 Alzheimer's Disease Facts and Figures).

Although the personal toll on AD patients can be devastating, and the financial impact of this disease continues to escalate, there may be hope on the horizon.

The key may be **calcium**, which plays a critical role in brain function. The dysregulation of calcium can in turn wreak havoc on brain function and is now clearly implicated in the development and progression of Alzheimer's disease and dementia.

How does calcium impact brain cell function?

It was first proposed more than 20 years ago that altered calcium regulation might play a role in normal brain aging, and in the development of Alzheimer's disease and dementia (Khachaturian, 1987; Thibault, Gant & Landfield, 2007; Green & LaFerla, 2008). Calcium is vital to many aspects of brain physiology, including growth, learning and memory. Specifically, it is integrally linked with neuroplasticity — the impressive ability of the brain to change its structure and function resulting from input and signals from the environment. Calcium serves as a critical intracellular messenger conveying these signals. In response to stimulatory signals from the environment, both electrical and chemical in nature, calcium ions flow through specialized channels in the plasma membrane of cells and then communicate with neurotransmitters to generate an appropriate response to the original signal. Calcium also works in a similar fashion to mediate signals necessary to help the brain recover in response to cell death and injury.

Several different pathways involving calcium are crucial to neuronal cell health. For example, calcium has been shown to activate families of cysteine proteases, which are enzymes that break down other proteins. The particular cysteine proteases in this case are calpains and caspases, which degrade a variety of substrates including cytoskeletal proteins, membrane receptors and other metabolic enzymes. Calpains may play an important role in the triggering

cellular signaling sequences that lead to apoptosis, or programmed cell death, by virtue of their ability to activate caspases that destroy layers of brain cells (Mattson, 2007).

Calcium homeostasis — the relative state of equilibrium associated with calcium levels in the brain — plays a crucial role in vital brain cell functions. For example, it can affect gene transcription, which is the transfer of genetic information from DNA to RNA allowing genes to produce proteins (Stutzmann, 2007).

Calcium also affects ion gradients, which are formed when there is a differing concentration of ions (high versus low) on either side of a membrane (Mattson, 2007). This difference results in potential energy, which can be used for critical chemical reactions needed for normal cell homeostasis. Ion gradients across brain cell membranes are critical to healthy cell function and rely on calcium levels. Specialized proteins present in the plasma membrane of these cells help to generate these gradients, and are sensitive to calcium concentrations as well.

Figure 1

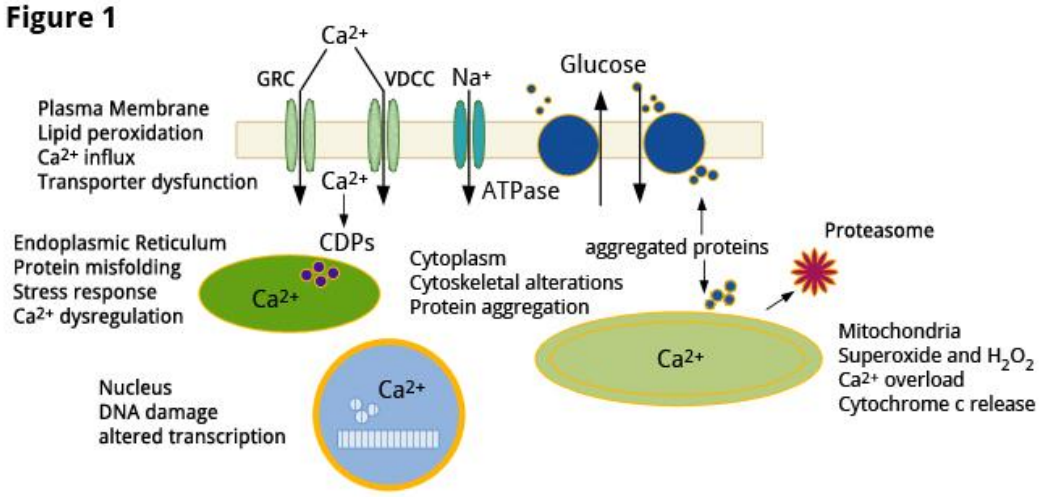

Fig. 1

There are several disrupted sub cellular systems involved in the disruption of neuronal Ca2+ homeostasis in aging and neurodegenerative disorders. Excessive Ca2+ influx through glutamate receptor channels and voltage-dependent Ca2+ channels is caused by age-related oxidative stress and other disease-specific mechanisms. This action also cause accumulation of Ca2+ within the cell. Perturbed Ca2+ homeostasis may also be triggered endoplasmic reticulum stress and mitochondrial dysfunction. Excessive amounts of Ca2+ within the neuron can cause dysfunction of several cellular processes. Alterations in Ca2+ homeostasis may also adversely affect synaptic plasticity, degeneration and death of neurons.

Studies of normal physiological activity have demonstrated that the concentration of intracellular calcium in neurons increases for a brief period of time (typically seconds to a few minutes) when stimulated, yet this has no adverse effects on such cells.

However, in the last two decades, evidence has accumulated showing that in pathological or disease states, as well as in normal cellular aging, the ability of neurons to control calcium fluxes and then recover from a transient increase becomes compromised (Thibault et al., 2007). Such studies were the first to link fluctuations in calcium levels in the brain with neurodegeneration.

Linking calcium dysregulation to Alzheimer's disease

More than 20 years of research have resulted in a solid body of evidence linking calcium and neuronal cell health. Given that the dysfunction and death of brain cells are hallmarks of AD, it is understandable that scientists have postulated that calcium dysregulation may be a significant factor in the

development of Alzheimer's. Scientific data to support this idea has expanded at an exponential rate.

In the mid-1980s, a hypothesis emerged describing how calcium overload might serve as a critical assault on brain neurons and effectively kill them off in patients with AD (Khachaturian, 1987). A series of studies comparing brain cells from aging rodents with those of younger animals supported this idea. Since then, however, researchers began to focus more attention on beta amyloid plaques and their role in neuronal degeneration. Evidence suggests that increased levels of beta amyloid plaques are toxic in brain cells. Moreover, tangles of beta amyloid protein have been identified in the brains of deceased Alzheimer's patients (Marx, 2007).

Even though cognitively normal adults may still exhibit substantial levels of beta amyloid plaques, and beta amyloid plaques are not typically detected until the middle to advanced stages of AD, a hypothesis emerged that the buildup of such plaques can contribute to Alzheimer's (Stutzmann, 2007).

Analyses of brain tissue from patients with neurodegenerative diseases first revealed evidence that changes in cellular calcium homeostasis also appear to contribute to the neurodegenerative process (Murray, Landsberg, Williams, Esiri, & Watt, 1992). Much of this work, published in the early 1990s, demonstrated that measurable amounts of free and protein-bound calcium, as well as the activity of calcium-dependent proteases, are increased in neurons showing amyloid plaques compared with clean, plaque-free neurons in brain tissue from AD patients (Murray et al., 1992; Nixon, 2003).

Seminal experiments involving artificial membranes designed to resemble the cell membrane were exposed to beta amyloid, and the protein formed very specific channels. These channels only permitted the flow of positively charged molecules, such as calcium, thereby increasing calcium concentrations in the cells (Arispe, 1993). Since those groundbreaking results were published, numerous laboratories have provided further evidence to support the idea that calcium disturbances underlie beta amyloid's toxic effects and lead to neuronal cell death.

Alzheimer's disease research has gained impressive momentum since the connection between calcium and beta amyloid was initially discovered. In 2008, a number of seminal studies made a strong case for incriminating altered calcium levels in the brain as a real culprit in the development of AD (Dreses-Werringloer et al., 2008; Cheung et al., 2008; Kuchibhotla et al., 2008; Rybalchenko, Hwang, Rybalchenko & Koulen, 2008). One study identified a gene specifically associated with late onset, sporadic AD (SAD) that disrupts a previously uncharacterized brain calcium channel. Individuals who carry one copy of the gene are at least 44 percent more likely to develop AD. Perhaps most importantly, the identification of this gene, and the protein which it encodes,

represents a product that could be targeted for future AD drug discovery and therapy (Dreses-Werringloer et al., 2008).

Another growing area of research involves the connection between Alzheimer's disease and the family of enzymes called presenilins. Presenilins were first identified in 1995 and found to be multi-membrane-spanning proteins, predominantly localized to the endoplasmic reticulum, an internal structure within the cell that forms an interconnected network of tubes and vesicles (Sherrington et al., 1995; Levy-Lahad et al., 1995).

As ion channels spanning a membrane, presenilins serve as gatekeepers for the amount of calcium that can flow in and out of a cell. Therefore, presenilins are directly involved in the overproduction of beta amyloid plaques that clog up brain cells, and may result in cell death in AD and dementia patients (Green & LaFerla, 2008).

Figure 2

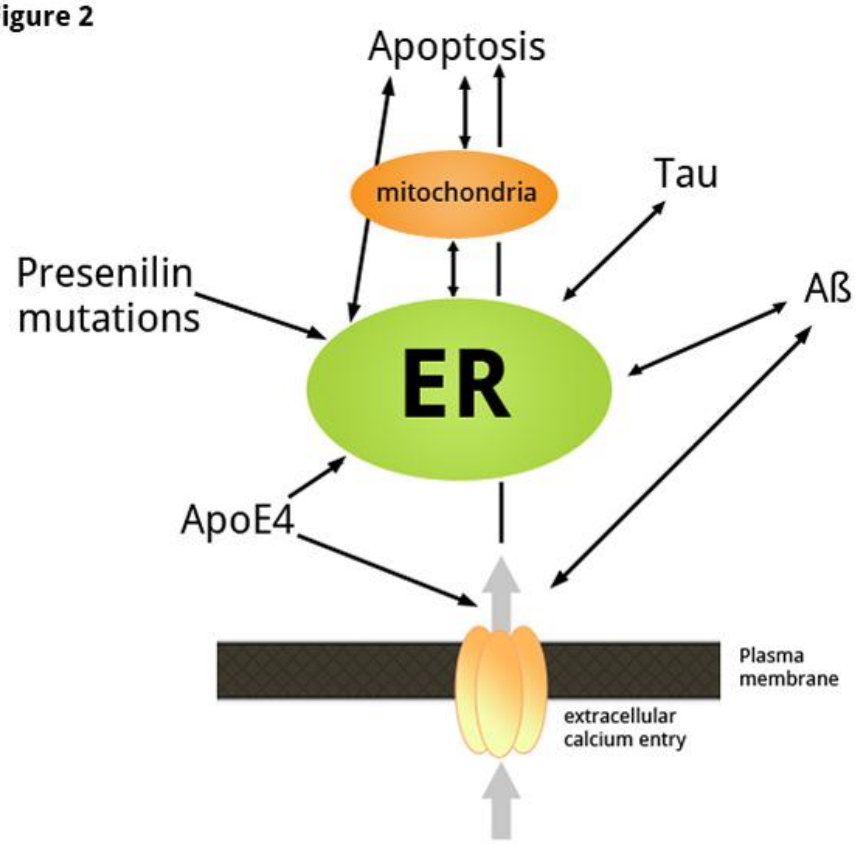

Fig. 2

Several pathways involve calcium in the early pathogenesis of Alzheimer's disease (AD). Calcium can trigger cellular degeneration in several ways as illustrated: extracellular calcium entry through the plasma membrane or intracellular release from the stores within the endoplasmic reticulum (ER) may have the ability to trigger the start of many of the principal features of AD. Many of these same features can trigger even further calcium release, supporting a pathological cycle. Disruptions in calcium-signaling may be present long before the onset of measurable symptoms, and slowly contribute to the accumulating cellular issues over the life of a person. Eventually the cell's compensatory mechanisms are overwhelmed and the cell degenerates.

The majority of cases of Alzheimer's disease occur spontaneously in individuals after age 60. However, approximately 10 percent of cases are inherited and can develop decades earlier. Known as familial Alzheimer's disease (FAD), these cases are also classified as early onset AD. Familial Alzheimer's disease has been shown to be linked to a mutation in the amyloid precursor protein, which then leads to an aggregation of sticky clumps of amyloid beta protein in the brain.

The recent discovery of presenilin mutations further implicates specific gene mutations in the development of FAD.

Solid research has linked mutant presenilin expression with an exaggerated intracellular calcium release in several model systems, including cells from FAD patients. This evidence suggests that the sustained disruption of intracellular calcium signaling may play an early role in the development of AD. One group of researchers found that biochemical interactions of mutant presenilin with a specific intracellular calcium release channel, the inositol trisphosphate receptor, profoundly increased channel activity (Cheung et al., 2008). These findings help explain how exaggerated calcium response is generated in cells exposed to normal stimulation, yet can cause low-level calcium signaling in unstimulated cells. The researchers went on to show that this enhancement of channel activity was directly involved in mutant presenilin-mediated beta amyloid generation, a telltale aspect of AD (Cheung et al., 2008).

Current treatments for Alzheimer's disease

The morphological and biochemical signatures underlying cell death in AD point to apoptosis, or programmed cell death. Noteworthy is the fact that **reductions in calcium-binding proteins** leading to unregulated calcium levels ultimately trigger the apoptotic signaling pathway (Stutzmann, 2007). These observations are important to the field of AD research, as they provide unique molecular insights into the calcium dysregulation hypothesis of AD pathogenesis. They also raise the promising possibility of novel targets for therapeutic intervention.

There are currently two types of treatment available for the management and treatment of AD. Each type works differently in the brain and targets a different pathway involved in neuron function, yet neither type of treatment represents a true cure. Cholinesterase inhibitors work by helping to increase the amount of acetylcholine in the brain, a chemical that is important for memory and learning. There are currently four cholinesterase inhibitors approved by the Food and Drug Administration to treat AD: Cognex, Aricept, Exelon and Razadyne.

The other class of medications are called NMDA (N-methyl-D-aspartate) receptor antagonists, or glutamate pathway modifiers. They work by interfering with the neurotransmitter glutamate, a molecule that normally targets the NMDA receptor and activates it by binding the receptor. Activation of the NMDA receptor through binding it sets off a cascade of reactions and signals in brain cells that, like the acetylcholine pathway, are critical in the processes of learning and memory.

By targeting NMDA receptors and binding to them in brain cells, NMDA receptor antagonists block the activity of glutamate. Glutamate is considered a

culprit in AD patients because when present at excessive levels, it overstimulates and effectively kills off brain cells.

The only drug in this class currently approved to treat the cognitive symptoms of moderate to severe AD is Namenda (memantine). Some studies have provided promising evidence that Namenda is effective in treating moderate to severe AD. However, a report published in the online edition of “Archives of Neurology” in April 2011 dashed many hopes with the news that Namenda appears to be ineffective in treating individuals with mild stages of AD (Schneider, Dagerman, Higgins, & McShane, 2011).

Future directions involving calcium-binding proteins show promise

With a mounting body of evidence indicating the key role of calcium regulation in neuronal cell health, it is no surprise that some of the more promising lines of research aimed at effectively treating AD are linked to controlling calcium flux in brain cells.

A novel strategy for controlling unregulated calcium levels in neuronal cells involves calcium-binding proteins. Studies have demonstrated that in cells with decreased levels of calcium-binding proteins, calcium homeostasis is adversely affected. This class of proteins has been shown to be vital in the regulation of calcium levels in certain cell types and is naturally depleted during the aging process. If intracellular calcium levels could be more tightly controlled through the activity of calcium-binding proteins, it is possible that neurodegenerative diseases like AD might be more effectively treated.

Apoaequorin is a calcium-binding protein originally discovered in a certain species of jellyfish many decades ago (Shimomura, Johnson, & Saiga, 1962; Shimomura, 2005; Inouye et al. 1985). Quincy Bioscience, headquartered in Madison, Wis., is actively focused on the discovery, development and commercialization of this promising molecule (Prevagen). In a fruitful partnership with research scientists at the University of Wisconsin-Milwaukee, the company has published several studies demonstrating the neuroprotective effects of apoaequorin (Detert et al., 2006).

At the same time Quincy Bioscience is focused on developing the molecule into an oral ingestible with the long-term goal of designing an over-the-counter supplement to enhance cognitive functioning, such as memory, in the aging population. In fact, Quincy Bioscience led a compelling Madison Memory Study showing the beneficial effects of apoaequorin on cognitive functioning in older adults. It presented the study findings at the 2011 Alzheimer’s Association International Conference (AAIC), the world’s leading forum on dementia research (Underwood, Sivesind, Gabourie, & Lerner, 2011). A total of 218 adults with self-reported memory concerns, aged 40 to 91 years, were enrolled in the study and

randomly assigned to receive either a daily dose of oral apoaequorin or a placebo. Participants receiving the calcium-binding protein not only tolerated the supplement well, but they also demonstrated a significant improvement in scores that measured functioning, learning and short-term memory. Current studies are aimed at eventually making apoaequorin available for the treatment of patients with AD.

In a comprehensive review of the state of the field of research linking calcium to AD (Green & LaFerla, 2008), co-authors Kim Green and Frank LaFerla concluded: "For the first time since the original calcium hypothesis of AD was proposed, we have functional and genetic data that push calcium to the crux for the sporadic version of the disease and highlight several potential new therapeutic targets for both the prevention and treatment of the disease. What is interesting is that the crucial risk factor for SAD, aging, also involves significant changes in calcium homeostasis, which could contribute to the initiation of the disease."

Investigations have increasingly focused on calcium and calcium-related cell-signaling disruptions involved in the onset and progression of AD. There is a growing interest in examining the therapeutic implications of normalizing disruptive calcium signaling and determining if this strategy may reduce the pathogenesis of AD. The potential of calcium-binding proteins to achieve normal and healthy calcium levels is certainly worth further examination.

There is tremendous hope and promise that apoaequorin will prove to be a successful example in this challenging scientific and clinical pursuit.

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