Stabilizing RyR2

By Lauren Martz, Staff Writer

A group at **Columbia University** has found that preventing the leak of calcium ions from ryanodine receptors could help treat post-traumatic stress disorder, and a French team has found that the strategy could be effective for Alzheimer's disease.[1,2] Based on these findings, **Armgo Pharma Inc.** is already pursuing the first indication and is considering testing its compounds in Alzheimer's disease.

Ryanodine receptor–operated calcium channels are one of the ways that calcium ions are released from the endoplasmic reticulum. Previous studies have implicated disruptions in calcium homeostasis in conditions ranging from cardiovascular disease to neurodegeneration.[3,4]

In 2004, Armgo was founded based on work from Columbia that showed the destabilization of ryanodine receptors in skeletal and cardiac muscle cells causes a calcium leak into the cytoplasm.[5] The group, led by Andrew Marks, found that ryanodine receptors in healthy cells are stabilized by FK506 binding protein 1B 12.6 kDa (FKBP1B; calstabin 2) and that dissociation of ryanodine receptors from calstabin 2 occurs in various diseases and causes calcium leak.

The Columbia team developed Rycals, which are compounds that promote the association between ryanodine receptors and calstabin 2 to prevent calcium leak. Armgo has a library of Rycals, and the company's most advanced candidate is in Phase II testing to treat heart failure.

Marks, chair of the Department of Physiology and Cellular Biophysics and founding director of the Clyde and Helen Wu Center for Molecular Cardiology at Columbia University College of Physicians and Surgeons, suspected that ryanodine receptors may be involved in pathologic mechanisms outside the cardiovascular space because they also are found in neurons. Thus, Marks and colleagues set out to determine whether ryanodine stabilization could improve stress-induced cognitive dysfunction.

The team used mice subjected to chronic restraint stress to model chronic stress–induced cognitive dysfunction such as post-traumatic stress disorder (PTSD). Stress caused alterations to ryanodine receptor 2 (RyR2), including hyperphosphorylation by protein kinase A (PKA) that ultimately depleted calstabin 2 and resulted in RyR2 destabilization and increased intracellular calcium levels caused by RyR2 channel leakage.

A brain-penetrating Rycal prevented these stress-induced effects, whereas a non–blood brain barrier (BBB)-penetrating Rycal did not. The BBB-penetrant Rycal was shown to reverse cognitive deficits by increasing learning, memory and spontaneous exploring and decreasing anxiety.

Knockout of the PKA phosphorylation site on RyR2 prevented the effects of chronic stress, confirming that the therapeutic effects of the Rycal were due to receptor stabilization.

Results were published in *Cell*.

Marks told *SciBX* that Columbia has patented the use of Rycals for PTSD and has licensed the IP to Armgo.

Armgo president and CEO Sapan Shah said the company is "trying to optimize candidates with good CNS penetration now for the treatment of stress-induced PTSD and Alzheimer's disease." He said the company is looking for partners in the CNS space.

Counting Rycals

Shah noted that the use of Rycals in neurological diseases could extend well beyond PTSD. For example, a 1994 paper by researchers at **The Rockefeller University** linked calcium dysregulation in neurons to Alzheimer's disease (AD).[6]

Now, Frédéric Checler, research director at **Institut National de la Sante et de la Recherche Medicale** (INSERM), and colleagues have found that RyR2 channel leaking occurs in hippocampal neurons during AD and that blocking the calcium release decreases levels of β-amyloid (Aβ) and cognitive deficits associated with the disorder.[2]

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> ***—Sapan Shah, Armgo Pharma Inc.***

The team included researchers from the **University Paris Descartes**, the **University of Nice Sophia Antipolis**, the **Italian Institute of Technology** and **Albany Medical College**.

In neuroblastoma cells overexpressing amyloid precursor protein (APP) and in a transgenic mouse model of AD, calcium release and RyR2 expression were both upregulated.

Also in APP-overexpressing neuroblastoma cells, using dantrolene to block ryanodine receptor decreased ryanodine receptor–dependent calcium release, levels of β-amyloid 42 and levels of proteolytic fragments from APP cleavage compared with using vehicle control.

In the transgenic AD mice, chronic administration of dantrolene after the start of AD pathology decreased the density of Aβ plaques compared with vehicle control administration. The generic muscle relaxant also increased learning and memory performance affected by mutant APP in the mice.

"*The Journal of Neuroscience* paper is exciting because it links ryanodine receptor–mediated calcium leak, elevated cytosolic calcium and β-amyloid production. This concept is promising as it could lead to a disease-modifying therapy for Alzheimer's. Current therapeutics for the disease such as acetylcholinesterase inhibitors don't address the underlying cause of disease," said Shah. "This establishes a link to another pathway to address the disease mechanism and, if effective,

could perhaps be a way to stem disease progression in addition to improving learning and memory."

Grace Stutzmann told *SciBX* that RyR2 stabilization affects the disease process earlier than many other AD targets. She is an associate professor in the Department of Neuroscience at the **Rosalind Franklin University of Medicine and Science and Chicago Medical School**.

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> ***—Grace Stutzmann, Rosalind Franklin University of Medicine and Science and Chicago Medical School***

"This approach is in contrast to existing strategies that focus on clearing late-stage features of AD, such as β-amyloid, which do not have a clear correlation with the cognitive deficits associated with AD," said Stutzmann. "It is not shocking that multiple clinical trials that employed approaches clearing late-stage features have been disappointing. Clearly an entirely new strategy is needed, and normalizing neuronal calcium is a logical approach."

Checler told *SciBX* that the next steps for his work include "development of a dantrolene-like compound with high specificity and affinity for ryanodine receptors and bioavailability."

Stutzmann said completely blocking ryanodine receptors is not desirable.

"My thought would be to establish that targeting calcium dysregulation has positive effects on early AD or mild cognitive impairment patients and to work on developing analogs that work by stabilizing calcium channels," she said. "We don't want to completely block RyR2-mediated calcium release; we just want to prevent the excess endoplasmic reticulum calcium release."

Shah agreed. "Dantrolene is a good research tool but may not be suitable for long-term chronic use in Alzheimer's patients. It is likely not a viable treatment because you don't want to block ryanodine receptor function completely, which may result in significant side effects or toxicities after long-term use. An optimal treatment would return ryanodine receptor to its normally functioning and nonleaky state."

Checler said he was not aware of Armgo's compounds but that it is theoretically possible they could elicit beneficial effects in AD.

He said his findings have not been patented and are not available for licensing.

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