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Ryanodine Receptor Patents

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Abstract

Research over the past two decades has implicated dysfunction of the ryanodine receptor (RyR), a Ca²⁺ release channel on the sarcoplasmic reticulum (SR) required for excitation-contraction (EC) coupling, in the pathogenesis of cardiac and skeletal myopathies. These discoveries have led to the development of novel drugs, screening tools, and research methods. The patents associated with these advances tell the story of the initial discovery of RyRs as a target for plant alkaloids, to their central role in cardiac and skeletal muscle excitation-contraction coupling, and ongoing clinical trials with a novel class of drugs called RycalsTM that inhibit pathological intracellular Ca²⁺ leak. Additionally, these patents highlight questions, controversies, and future directions of the RyR field.

Keywords

Ryanodine; calcium; EC-coupling; myocardium; skeletal muscle; sarcoplasmic reticulum (SR); calcium leakage; leaky SR; Rycal; JTV519; S107; heart failure; malignant hyperthermia; arrhythmias; patents

INTRODUCTION

Cardiac and skeletal muscle contractions are activated by depolarization of the sarcolemma and release of intracellular Ca²⁺ stores from the SR into the cytosol. Cytosolic Ca²⁺ binds to troponin C resulting in shortening of myofilaments and muscle contraction. This process, known as EC coupling requires RyR to release SR Ca²⁺ [1].

Skeletal and cardiac muscle EC-coupling are analogous but there are important differences. In brief, depolarization of skeletal myocytes begins with binding of acetylcholine to receptors at the neuromuscular junction, leading to Na⁺ influx, and activation of the L-type Ca²⁺ channel. The skeletal muscle RyR1 isoform releases SR Ca²⁺ due to activation by a

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CONFLICT OF INTEREST

Andrew R. Marks is a consultant for and has ownership in ARMGO Pharma, Inc., a company that is developing ryanodine receptor targeted therapeutics.

direct protein-protein interaction with the L-type Ca^{2+} channel [2]. In the heart, a depolarizing Na^{+} current activates Ca^{2+} influx via the L-type Ca^{2+} channel which in turn activates the RyR2 isoform via Ca^{2+} -induced- Ca^{2+} release [3].

While most research has focused on RyR1 in skeletal muscle and RyR2 in cardiac muscle, these RyR isoforms, along with RyR3, have been identified in many other tissues including central nervous system, smooth muscle, T and B-lymphocytes, and pancreatic β -cells [4].

RyR is a homotetramer comprised of four approximately 5000 amino acid, 565 kDa monomers. The channel has a large cytosolic domain, accounting for 90% of the primary structure. Since RyR1 and RyR2 were cloned 23 years ago [5, 6] many binding and modulator proteins, including FKBP12.6 (FKBP12 in RyR1), protein kinase A (PKA), PDE4D3, calmodulin, and Ca^{2+} /calmodulin dependent kinase (CaMKII) have been identified as part of the RyR2 macromolecular complex [1, 7].

INSECTICIDE RELATED PATENTS

RyR was named based on its purification from skeletal muscle lysate using the high affinity plant alkaloid ryanodine [8]. When bound to RyR, ryanodine locks the channel in a half open state resulting in depletion of Ca^{2+} from the SR and interruption of EC-coupling. This explains the historical use of extracts from the *Ryania* plant family by natives of South and Central America as poison for arrowheads because the release of SR Ca^{2+} via the locked open RyR channels causes tetany [9].

The observation that insects would not feed on plants from the *Ryania* family suggested the presence of a natural insecticide [9]. The insecticidal property of the *Ryania speciosa* Vahl plant was patented in 1946 based on work performed in cooperation with the Department of Entomology of Rutgers University [10] (Table 1). In 1948, ryanodine, the compound responsible for the insecticidal activity of the *Ryania speciosa* Vahl, was isolated and characterized. The purified alkaloid demonstrated 700 times the insecticidal property of the stem wood extract [11]. However, unsuccessful attempts to produce ryanodine as an economically viable insecticide with minimal mammalian toxicity resulted in reduced interest in RyR as an insecticide target [12].

Renewed interest came in July 2005 with the unveiling of Flubendiamide, a phthalic acid diamide, at the International Conference on Pesticides in Kuala Lumpur [13]. Flubendiamide, produced in a collaboration between Nihon Nohyaku [14, 15] and Bayer CropScience, selectively activates insect RyR with a K_D of 4.7nM by binding within the transmembrane domain (amino acids ~4100–5000) [16], at a site distinct from that of ryanodine [17]. Flubendiamide was demonstrated to selectively inhibit insect RyR without any appreciable effects on mammalian RyR1, RyR2, or RyR3 [18].

Following the announcement of Flubendiamide, DuPont reported a novel class of insecticides, the anthranilic diamides [19], which exhibit high potency for insect RyR and low potency for mammalian RyR. The discovery of the phthalic acid and anthranilic diamides which share many similar structural and biochemical properties [20], resulted in the creation of group 28 of insecticides (RyR modulators) by the Insecticide Resistance Action Committee (IRAC) [21].

Efforts are underway to develop insecticidal agents that selectively target insect RyR based on sequence analysis and characterization of insect ryr gene product. Insect species being targeted include lepidopteran tobacco budworm (*Heliothis virescens*), corn plant hopper (*Peregrinus maidis*), fruitfly (*Drosophila melanogaster*) [22], and silkworm [23].

SKELETAL MUSCLE RELATED PATENTS

Patients with malignant hyperthermia (MH) exhibit catastrophic, hyperactive skeletal muscle metabolic activity in response to fluorinated inhalation anesthetics. Without rapid recognition of the disease and administration of dantrolene, mortality secondary to hyperthermia, as high as 43°C, and complete circulatory collapse is high [24]. MH follows an autosomal dominant pattern of inheritance with an estimated incidence of susceptibility of 1 in 2000–3000 [25]. The absence of basal overt signs or symptoms in susceptible individuals has made it difficult to anticipate a patient's predisposition to MH although obtaining a careful family history is critical [24].

The first generation of clinical MH tests took advantage of the enhanced sensitivity of skeletal muscle fibers isolated from MH patients to contract in response to caffeine and halothane. However, these studies required a large, fresh biopsy sample from patients. The invasive and expensive nature of these tests precluded their use as a viable screening test for patients suspected of having MH [26] (Table 2).

The presence of a gain-of-function defect in RyR1 to explain the molecular mechanism of MH was initially proposed based on higher rates of Ca^{2+} -induced- Ca^{2+} release [27] and higher ^3H -ryanodine binding [28] at low $[\text{Ca}^{2+}]$ in MH skeletal muscle preparations. Gene sequencing and association studies eventually linked MH to mutations in RyR1 [29]. One of these mutations, substitution of arginine 615 with cysteine, created a novel trypsin cleavage site in RyR1 [30, 31]. The presence of a prominent 86 kDa band in RyR1 from MH patients treated with trypsin, compared to a 99 kDa band in normal patients enabled protease cleavage patterns to be used as a screening test for MH susceptible patients [32, 33].

Diagnostic tests taking advantage of novel restriction fragments in MH cDNA have also been described [34]. These tests have been patented as diagnostics for MH in swine, as porcine MH is associated with inferior quality pork and high mortality [35].

Peripheral lymphocytes isolated from MH patients exhibit exaggerated intracellular Ca^{2+} release responses, similar to MH skeletal muscle, when exposed to RyR activating agents. Adenosine and inosine are metabolic byproducts of ATP production [36]. Levels of these molecules purportedly increase during an MH episode secondary to rapid metabolic activity. Measuring adenosine and inosine levels in lymphocytes exposed to RyR activating agents has been proposed as a screening test for MH [37]. The observation that peripheral lymphocytes express functional RyR1 presents an opportunity to use these cells for diagnosing RyR related pathology [38].

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder characterized by progressive muscle weakness and wasting. The disease affects approximately 1 in 3500 boys and mortality prior to age 30, secondary to respiratory failure, is high [39]. Studies of non-necrotic skeletal muscle fibers from patients with DMD revealed evidence of intracellular Ca^{2+} overload [40]. Elevation of $[\text{Ca}^{2+}]_{\text{cyt}}$ leads to activation of Ca^{2+} -dependent neutral proteases (calpains) and degradation of muscle fibers [41]. While cytosolic Ca^{2+} overload has been attributed to impaired sarcolemmal integrity, plasma membrane Ca^{2+} “leak” channels [42], and microscopic plasma membrane tears [43], a role for dysfunctional RyR1 has also been reported [44].

Initial evidence linking SR Ca^{2+} leak to elevated $[\text{Ca}^{2+}]_{\text{cyt}}$ in DMD came from the observation that Ca^{2+} spark rate was increased in dystrophic skeletal muscle [45]. Ca^{2+} sparks are low amplitude, transient SR Ca^{2+} release events that represent spontaneous opening of RyR clusters. Ca^{2+} spark rate is related to RyR open probability, the chance that a channel will spontaneously open at any given moment. This relationship is based on the

observation the spark rate increases following exposure to substances that sensitize RyR to $[Ca^{2+}]_{cyt}$ [46]. SR Ca^{2+} leak refers to SR Ca^{2+} release while a muscle is at rest.

Neuronal-nitric oxide synthase (nNOS), the predominant skeletal muscle NOS, localizes to the plasma membrane and is downregulated in DMD [47]. In contrast to nNOS, inducible NOS (iNOS) co-localizes with RyR1 [44] and is increased in DMD [48]. S-nitrosylation of RyR by nitric oxide synthase (NOS) increases channel activity [49, 50].

S-nitrosylation mediated activation of RyR1 is associated with disruption of the binding of the stabilizing subunit cal-stabin1 (FKBP12) to RyR1 [51]. Calstabin1 is a small 12 kDa prolyl isomerase which binds to each RyR1 monomer and stabilizes the closed state of the channel [52].

It has been shown that increased iNOS expression and S-nitrosylation of RyR1 causes calstabin1 depletion from the channel macromolecular complex resulting in pathologic SR Ca^{2+} leak. S107, a cell permeable small molecule derived from the 1,4-benzothiazepine, JTV519, prevents calstabin1 depletion from the RyR1 macromolecular complex and prevents Ca^{2+} leak via the channel [53]. Treating muscular dystrophic (*mdx*) mice with S107 results in improved muscle specific force, grip strength, reduced fatigue and improved exercise capacity [44]. Pharmacologic inhibition of RyR1 mediated SR Ca^{2+} leak is a novel therapy for improving muscle function in patients with DMD [54].

Intense prolonged exercise (e.g. marathon running) can result in muscle damage. PKA phosphorylation and S-nitrosylation of RyR1, and depletion of calstabin1 from RyR1 are associated with prolonged strenuous exercise [53]. It has been proposed that acute PKA phosphorylation of RyR1 during exercise increases SR Ca^{2+} release and muscle force generation [52] but that chronic phosphorylation [55], and S-nitrosylation, results in pathologic SR Ca^{2+} leak [53]. Mice subjected to an intense three-week exercise regimen performed better and had less evidence of muscle damage when treated with S107 compared to vehicle treated controls [53].

Chronic conditions such as heart failure and age-related loss of muscle function [56] may share similar pathophysiologic components as DMD and strenuous exercise. Pharmacological inhibition of RyR1 mediated SR Ca^{2+} leak could provide muscle protection and improved exercise capacity to patients with these conditions [54, 57, 58].

Myasthenia gravis is an autoimmune disease characterized by muscle weakness secondary to destruction of nicotinic acetylcholine receptors. In a recent study patients with auto-antibodies targeting RyR1 had significantly worse EC-coupling impairment than those without these antibodies [59]. Methods for detecting RyR1 antibodies may serve as a prognostic indicator for patients with myasthenia gravis [60].

CARDIAC RELATED PATENTS

According to the American Heart Association there were over five million Americans suffering from heart failure in 2008. Patients with heart failure have poor cardiac function resulting in shortness of breath and decreased exercise capacity. There are four New York Heart Association heart failure classes ranging from class I (limitation on physical activity) to class IV (unable to perform any physical activity without discomfort). Patients hospitalized with heart failure have 43.5% mortality by five years [61].

The β -adrenergic receptor and angiotensin/aldosterone system are key targets of FDA approved pharmacologic therapies for heart failure [62]. However, despite these treatments mortality from heart failure, secondary to pump failure and sudden cardiac death, remains

high. In 2008 heart failure was responsible for 56,830 deaths and over one million hospitalizations in the United States [61].

Common causes of heart failure in the western world include cardiac ischemia, hypertension, and valvular disease. However, mortality in heart failure, regardless of etiology, correlates with serum norepinephrine (NE) levels [63]. The mortality benefit of β -blockers in heart failure supports these findings [62]. Molecular mechanisms linking the therapeutic efficacy of β -blockers in heart failure include: increasing β -adrenergic receptor density, slowing heart rate thereby reducing negative inotropy, and allowing for increased SR Ca^{2+} load [64].

Depletion of SR [Ca^{2+}] in cardiomyocytes from heart failure patients [65] was initially attributed to decreased expression of the SR Ca^{2+} reuptake pump and decreased SR Ca^{2+} loading [66].

A report by Marx *et al.* in 2000 showed for the first time that RyR2 from heart failure patients and from animal models of heart failure are “leaky” due to PKA hyperphosphorylation at Ser2808 and depletion of the channel stabilizing subunit calstabin2 (FKBP12.6) [67]. PKA hyperphosphorylation refers to chronic phosphorylation of all four RyR2 subunits. RyR2 channels from failing hearts were pathologically sensitized to activation by low levels of cytosolic Ca^{2+} and exhibited increased open probability at diastolic [Ca^{2+}] under conditions where channels from normal non-failing hearts are tightly closed [67]. These findings suggested a model for heart failure progression in which leaky RyR2 channels deplete SR Ca^{2+} causing weakened cardiac muscle contraction and provide the trigger for fatal cardiac arrhythmias [67].

Supporting this model, cardiomyocytes isolated from mice engineered lacking calstabin2 exhibited an increased rate of arrhythmogenic delayed-after depolarizations, and the mice suffered from exercised induced sudden cardiac death [68].

The theory has recently evolved. PKA hyperphosphorylation of RyR2 is associated with S-nitrosylation and oxidation of RyR2, which renders the channels leaky [69].

Others have reported that S-nitrosylation of RyR2 stabilizes the channel and that decreased S-nitrosylation is responsible for RyR2 mediated SR Ca^{2+} leak. However, the data supporting this conclusion (conclusions from Fig. 2A of the referenced paper are based on a measurement of RyR2 nitrosylation by immunoblot with no control for the amount of protein loaded, making this impossible to assess [70]) Nevertheless, based on these findings agents that increase S-nitrosylation of RyR2 have been proposed to have therapeutic benefit in heart disease [71, 72].

The link between RyR2 mediated SR Ca^{2+} leak and arrhythmias is attributed to transient inward current (I_{ti}) generated by the Na^+ - Ca^{2+} exchanger (NCX). The NCX removes one Ca^{2+} ion in exchange for three Na^+ ions. This net inward depolarizing current increases the probability of delayed after-depolarizations and premature ventricular contractions (PVCs) [1].

The RyR2 SR Ca^{2+} leak hypothesis provides a novel understanding for the mechanism of β -blockers. In failing hearts RyR2 channels are PKA hyperphosphorylated in part due to a depletion of PDE4D from the RyR2 complex due to oxidation [69, 73]. By blocking activation of β -adrenergic receptors β -blockers prevent PKA hyperphosphorylation of RyR2 and SR Ca^{2+} leak. In fact, mice engineered with constitutively PKA hyperphosphorylated RyR2, (RyR2-S2808D), in which the serine that is phosphorylated by PKA (Ser2808) is replaced by an aspartic acid, are impervious to the therapeutic effects of β -blockers [69].

Repairing RyR2 mediated SR Ca^{2+} leak is a novel therapeutic approach for heart failure. The small molecule JTV519 was originally identified based on its ability to prevent myofibrillar overcontraction in a model of myocardial injury [74]. The cardioprotective effects of JTV519 were attributed to non-specific inhibition of Na^+ , Ca^{2+} , and K^+ currents [75] as well as to the activity of protein kinase C [76] and nitric oxide synthase [77]. However, subsequent studies showed that JTV519 inhibited the stress-induced dissociation of calstabin2 from the RyR2 macromolecular complex [78–81] (Table 3).

The therapeutic efficacy of JTV519 in heart failure was initially reported in a study where canines pretreated with the drug were protected from pacing induced heart failure [79]. In a mouse model of heart failure, calstabin2 deficient mice treated with JTV519 did not demonstrate improved myocardial function as observed in wild type mice [82]. Similarly, JTV519 reduced the incidence of sustained polymorphic ventricular tachycardia in wild type and heterozygous cal-stabin2 deficient mice but not homozygous calstabin2 deficient mice [78]. These experiments provided definitive evidence that the cardioprotective effect of JTV519 was due to its ability to stabilize binding of calstabin2 to RyR2.

Others have proposed that CaMKII phosphorylation of RyR2, independent of PKA phosphorylation and calstabin2 depletion from the channel, are responsible for myocardial dysfunction in heart failure. Furthermore, there is evidence that CaMKII is activated downstream of the β -adrenergic receptor, which could also explain the mechanism of action of β -blockers [83]. However, this hypothesis is challenged by data that mice engineered to lack the CaMKII phosphorylation site on RyR2 are not protected from ischemic heart failure progression [84]. Patents on heart failure therapies targeting CaMKII [85, 86] as well as those that stabilize the interaction between calstabin2 and RyR2 [87–90] cover both possibilities (PKA and CaMKII).

Others are developing methods to stabilize RyR2 in heart failure using small peptides. One sequence that has shown promise is a peptide corresponding to amino acids 2114 to 2149 of RyR2 [91]. Another peptide under development is one corresponding to the region of the L-type Ca^{2+} channel responsible for inhibiting RyR1 in skeletal muscle [92].

Some investigators have proposed that RyR2 is regulated by SR luminal $[\text{Ca}^{2+}]$ instead of cytosolic $[\text{Ca}^{2+}]$. They propose that arrhythmias occur when SR $[\text{Ca}^{2+}]$ reach above a threshold level. According to this hypothesis, known as store overload induced Ca^{2+} release (SOICR) [93, 94], compounds that reduce the sensitivity of RyR2 to SR $[\text{Ca}^{2+}]$ have therapeutic potential in heart disease [95].

Another approach for modulating RyR in SR Ca^{2+} leak related cardiac, brain, and vascular pathologies targets estrogen receptor (ER) mediated stimulation of RyR activity [96]. This proposal is based on a report demonstrating RyR co-localization with ER beta in the neuronal cell line HT-22, and that application of the soluble monomer of the long form ER beta results in increased RyR open probability [97].

Mice lacking phosphodiesterase (PDE4D), an enzyme that degrades cAMP causing deactivation of PKA, have PKA hyperphosphorylated RyR2 and develop cardiomyopathy [73]. Based on these findings, another approach for stabilizing RyR2 in heart disease would be to develop methods for increasing PDE4D activity in cardiomyocytes [98].

By stabilizing dysfunctional RyR2, JTV519 has been shown to prevent sudden cardiac death in mice with the autosomal dominant inherited arrhythmia catecholaminergic polymorphic ventricular tachycardia (CPVT). There are over 70 mutations in RyR2 that are associated with CPVT (a current list is available at <http://www.fsm.it/cardmoc>). Patients with CPVT are asymptomatic and exhibit normal ECGs at rest. However, during exercise these

individuals have a high risk for sudden cardiac death due to ventricular fibrillation (VF). Current FDA approved therapies include β -blockers and implantable cardioverter-defibrillators. Mice engineered with CPVT mutations in RyR2 exhibit decreased affinity of calstabin2 to RyR2 and suffer from exercise induced sudden cardiac death due to VF [68]. Pre-treatment with JTV519 prevents these arrhythmias [81]. This further demonstrates the anti-arrhythmic potential of drugs that stabilize the interaction of calstabin2 with RyR2 [99, 100]. Furthermore, the availability of murine CPVT models, mice harboring RyR2 mutations corresponding to human CPVT-linked mutations, will aid the development of drugs for targeting RyR2 related arrhythmias [101].

The non-specific effects of JTV519 on plasmalemmal ion channels led to the development of S107 [53]. S107 reduces RyR2 mediated SR Ca^{2+} leak and reverses the progression of heart failure in mice engineered with chronically PKA phosphorylated, nitrosylated and oxidized RyR2 [69]. A number of compounds, similar to S107, are being developed for clinical trials.

RyR2 isolated from patients with atrial fibrillation (AF), a chronic arrhythmia affecting over two million Americans [61], is PKA hyperphosphorylated and depleted of Cal-stabin2 [102]. Controversy exists whether CaMKII [103–105], or PKA, is the enzyme responsible for causing leaky RyR2 in AF. Furthermore, there is controversy whether or not depletion of calstabin2 from RyR2 is necessary for arrhythmogenesis [83]. There is, however, broad consensus that RyR2 mediated SR Ca^{2+} leak contributes to the pathogenesis of AF.

Given the lack of effective anti-arrhythmic agents for rhythm control, current AF therapy focuses on rate control as well as anti-coagulation to prevent thrombogenesis in the fibrillating atria. Therefore, targeting leaky RyR2 presents a novel therapeutic approach for treating this disease. Patents have been filed targeting both CaMKII [85] activity and stabilizing calstabin2 binding to RyR2 [99].

RyR2 dysfunction has also been linked to ischemia/ reperfusion injury [106] and pathologic cardiac hypertrophy [107]. Compounds, such as S107, which stabilize RyR2 have therapeutic potential in these diseases [108, 109].

Another therapeutic concept for treating heart disease is to use stem cells to build a custom cellular Ca^{2+} handling system. Using this approach RyR2, the SR Ca^{2+} uptake pump, and NCX levels could be optimally adjusted. The stem cells could then be implanted into a diseased heart where they would reproduce to form healthy myocardium [110].

BRAIN RELATED PATENTS

With clinical trials related to cardiac and skeletal muscle RyR pathologies in progress the upcoming area of excitement is nervous system pathology. Patents focused on targeting RyR in diseases ranging from neuronal ischemic reperfusion injury [111] to amyotrophic lateral sclerosis [112] and Huntington disease [113] have been filed (Table 4).

One promising area of RyR brain related research is the role of intracellular Ca^{2+} release in cognitive function. In one study, RyR3 deficient mice exhibited impaired maintenance of hippocampal long-term potentiation (LTP), a key component of memory formation [114]. RyR2 and RyR1 are also widely expressed in the hippocampus [115] and contribute to synaptic transmission [116] and memory formation [117]. The central role of the hippocampus in memory formation suggests that RyR mediated intracellular Ca^{2+} leak may contribute to the pathogenesis of cognitive dysfunction in diseases such as Alzheimers and Post-Traumatic Stress Disorder [118, 119].

The presence of all three human RyR isoforms in the brain has historically made it difficult to tease out the contribution of these channels to brain pathology. However, with time, the development of conditional knockouts of these three isoforms as well as better methods for localizing isoform expression to specific neuronal populations will help our understanding of RyR dysfunction in nervous system pathology [4] (Table 5).

CONCLUSIONS

Despite the fact that over 40 RyR related patents are currently filed, the central role that SR Ca^{2+} leak plays in human physiology is likely to lead to many more. Many of these patents target disease processes that were once considered to be irreversible. Now that these patents are heading towards clinical trials hope for many suffering patients may be just around the corner.

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Table 1**Insect Related RyR Patents**

Patent No.	Inventor/Assignee	Title	Description
US2400295	Folkers, Rogers and Heal	Insecticides	Extracts of alkaloids from the genus <i>Ryania</i> exhibit pronounced insecticidal activity
US6362369	Tohnishi, M, <i>et al.</i> / Nihon Nohyaku Co., Ltd. (Tokyo, JP)	Phthalic acid diamide derivatives fluorine-containing aniline compounds as starting material, agricultural and horticultural insecticides, and a method for application of the insecticides	Characterization of novel compounds for selectively targeting insect RyR
US7964204	Lahm, G.P., <i>et al.</i> / E.I. du Pont de Nemours and Company (Wilmington, DE)	Isoxazolines for controlling invertebrate pests	Characterization of novel compounds for selectively targeting insect RyR
US7498408 US8101715 US7655395 US7205147	Gutteridge, S, <i>et al.</i> / E. I. du Pont de Nemours and Company (Wilmington, DE)	Ryanodine receptor polypeptides	Analysis of ryr gene sequence from lepidopteran tobacco budworm (<i>Heliothis virescens</i>), corn plant hopper (<i>Peregrinus maidis</i>), fruitfly (<i>Drosophila melanogaster</i>) for the purpose of insecticides development
WO2007094408	Kyoto University	Silkworm RyR protein, cDNA thereof, recombinant vector, expression plasmid, cell showing constant expression, method of screening insecticidal component and method of screening substance reacting with silkworm RyR.	Analysis of ryr gene sequence from silkworm for the purpose of insecticide development

Table 2**Skeletal Muscle Related RyR Patents**

Patent No.	Inventor/Assignee	Title	Description
US5242801 US5462857	Campbell, Kevin University of Iowa Research Foundation	Diagnosing malignant hyperthermia susceptibility by detection of abnormal proteolytic enzyme digestion fragments of the ryanodine receptor	Diagnosing malignant hyperthermia susceptibility based on abnormal ryanodine receptor protease digestion fragments.
US5413907	The University of Toronto Innovations Foundation HSC Research and Development Limited Partnership The Toronto Hospital	Diagnosis for malignant hyperthermia	Using specific RyR1 cDNA as a probe for restriction fragment length polymorphism analysis in malignant hyperthermia
US7704990	Landry, D. <i>et al.</i> The Trustees of Columbia University In The City of New York	Agents for preventing and treating disorders involving modulation of the RyR receptors	Developing therapies for cardiac, skeletal, and neurologic pathologies resulting from RyR dysfunction
US8022058 US7879840 US20110172190	Marks, Andrew The Trustees of Columbia University In The City of New York	Agents for preventing and treating disorders involving modulation of the RyR receptors	Developing therapies for cardiac, skeletal, and neurologic pathologies resulting from RyR dysfunction
US7312044	Marks, Andrew The Trustees of Columbia University In The City of New York	Type 1 ryanodine receptor- based methods	Treating skeletal muscle disease by developing compounds to stabilize the association between calstabin1 (FKBP12) and RyR1
US6001976	University of Toronto Innovations Foundation and University of Guelph	Diagnosis for porcine malignant hyperthermia	Using ryr1 restriction endonuclease patterns for diagnosing malignant hyperthermia susceptibility in porcine
WO2000075658	Unifob, Stiftelsen Universitets- forskning Bergen	Detection of RyR1 antibodies	Detection of RyR1 antibodies from patient serum as a prognostic indicator for myasthenia gravis.
WO2010028372	The Henry M. Jackson foundation for the Advancement of Military Medicine, Inc.	Methods and kits for the diagnosis of ryr1-related diseases	Use of adenosine and inosine levels in lymphocytes treated with RyR activating agents for diagnosing MH.
WO2008144483	ARMGO Pharma	Agents for treating disorders involving modulation of ryanodine receptors	Treating RyR related pathologies including cardiac, muscular and cognitive disorders and diseases using derivatives of benzoxazepines, benzodiazepines and benzazepines.
WO2008064264	ARMGO Pharma	In vivo methods for identifying and screening compounds that modulate calstabin binding to a ryanodine receptor	In vivo methods for identifying compounds, which stabilize binding of calstabin2 to RyR2.
WO2012019071	Trustees of Columbia University in the City of New York	Methods of preventing and treating sarcopenia	Use of benzothiazepine, benzoxazepine, benzodiazepine or benzazepine related compounds for treating or preventing sarcopenia.
WO2008060332	Trustees of Columbia University in the City of New York	Methods for treating or reducing muscle fatigue	Use of RyR stabilizing drugs to treat, prevent or reduce muscle fatigue from strenuous exercise and secondary to a range of diseases.

Table 3

Cardiac Related RyR Patents

Patent No.	Inventor/Assignee	Title	Description
US7544678 US7718644	Marks, Andrew The Trustees of Columbia University In The City of New York	Anti-arrhythmic and heart failure drugs that target the leak in the ryanodine receptor (RyR2)	Developing anti-arrhythmic agents that function by stabilizing the interaction between RyR2 and calstabin2 (FKBP12.6).
US7704990	Landry, D. <i>et al.</i> The Trustees of Columbia University In The City of New York	Agents for preventing and treating disorders involving modulation of the RyR receptors	Developing therapies for cardiac, skeletal, and neurologic pathologies resulting from RyR dysfunction
US8022058 US7879840 US20110172190	Marks, Andrew The Trustees of Columbia University In The City of New York	Agents for preventing and treating disorders involving modulation of the RyR receptors	Developing therapies for cardiac, skeletal, and neurologic pathologies resulting from RyR dysfunction
WO2009026444	President and fellows of Harvard College	Ryanodine channel binders and uses thereof	Development of JTV-519 derivatives for treating heart failure, atrial fibrillation, or exercise-induced cardiac arrhythmias.
US7851589	Otsuka Pharmaceutical Co., Ltd.	Polypeptide derived from the ryanodine receptor that inhibits Ca ²⁺ leakage	Stabilizing RyR2 in heart disease using polypeptides corresponding to RyR2 amino acids 2114 to 2149.
US20050049198	The Australian National University	Method of modulating the activity of Ca ²⁺ channels in cardiac cells and reagents therefor	Modulating RyR2 using peptides corresponding to the region of the L-type Ca ²⁺ channel that inhibits RyR1
US20100316622	University of Miami	Diagnosis and treatment of cardiac disorders	Methods for enhancing S-nitrosylation of RyR2 in order to prevent heart disease
WO1999055317	The Johns Hopkins university	Treatment of advanced decompensated heart failure with nitric oxide donors	S-nitrosylation of RyR2 in order to improve SR Ca ²⁺ release and enhance inotropy.
US20120014924	The Regents of the University of California	Compositions and Methods for Improving the Functional Efficacy of Stem Cell-Derived Cardiomyocytes	Using stem cells to develop custom Ca ²⁺ handling systems, which can then be implanted into diseased myocardium
US7741529	Priori, Silvia G.	Transgenic animal model for catecholaminergic polymorphic ventricular tachycardia (CPVT) and use thereof	Using transgenic animals for developing therapies for catecholaminergic polymorphic ventricular tachycardia (CPVT) and RyR2 related arrhythmias.
WO2012019076	Trustees of Columbia University in the City of New York	Compositions and methods for preventing and treating cardiac ischemia/reperfusion injury	Development of compounds that stabilize RyR2 for the prevention of cardiac ischemia/reperfusion (I/R) injury.
WO2008021439	Trustees of Columbia University in the City of New York	Compositions and methods for treatment of cardiac hypertrophy	Development of compounds that stabilize RyR2 for the prevention of cardiac hypertrophy.
WO2007127145	Trustees of Columbia University in the City of New York	Methods and compositions for treatment of cardiac arrhythmia in non-human animals	Developing anti-arrhythmic agents, which function by stabilizing the interaction between RyR2 and calstabin2 (FKBP12.6) for non-human animals.
US20060293266	Marks, Andrew The Trustees of Columbia University in the City of New York	Phosphodiesterase 4D in the ryanodine receptor complex protects against heart failure	Treating RyR related cardiac, skeletal, and neurologic pathology by developing methods to deliver PDE4D to cells.
US6489125 US7393652	Marks, Andrew The Trustees of Columbia University In The City of New York	Methods for identifying chemical compounds that inhibit dissociation of FKBP12.6 binding protein from type 2 ryanodine receptor	Developing therapies for heart disease, which function by stabilizing the interaction between RyR2 and calstabin2 (FKBP12.6).

Table 4

Nervous System Related RyR Patents

Patent No.	Inventor/Assignee	Title	Description
WO2012037105	Trustees of Columbia University in the City of New York	Methods of treating, ameliorating or preventing stress-induced neuronal disorders and diseases	Stabilizing RyR Ca^{2+} leak for treatment of stress-induced cognitive dysfunction that occurs in situations such as substance abuse.
WO2008021432	Trustees of Columbia University in the City of New York	Compositions and methods for treatment of conditions affecting the nervous system	Stabilizing RyR Ca^{2+} leak for treatment and prevention of diseases, disorders, and conditions affecting the nervous system, such as neuropathies, seizures, and disorders affecting the cognitive functioning.
US6462066	University of South Florida	Method and composition for treatment of ischemic neuronal reperfusion injury	Antagonizing RyR3 as prophylaxis for ischemic optic neuropathy as well as other ischemic reperfusion neuropathies.
US20070049630	Allergan, Inc.	Method of using ryanodine receptor antagonists to treat amyotrophic lateral sclerosis	Use of RyR antagonist to treat amyotrophic lateral sclerosis by preventing neuronal injury or death
US20070066638	Allergan, Inc.	RyR blockers for treating pain	Blocking RyR to inhibit or prevent pain.
WO2001034633	Fred Hutchinson cancer research center	Methods for treatment of human Huntington's Disease and methods of screening for active agents	Therapies targeting RyR Ca^{2+} handling in the pathogenesis of Huntington's Disease.
US20060030548 US20040006124	Allergan, Inc.	Methods of using ryanodine antagonists in treating neural injury	Blocking RyR to prevent noxious action on nerve cells.

Table 5

Miscellaneous RyR Patents

Patent No.	Inventor/Assignee	Title	Description
WO2007143112	Trustees of Columbia University in the City of New York	Methods for diagnosing disorders and diseases associated with ryanodine receptors and methods for identifying compounds that affect ryanodine receptors	Using peripheral lymphocytes to diagnose RyR related pathologies
WO2008140592	ARMGO Pharma	Radioactively labeled 1,4-benzothiazepines and methods of screening for compounds that bind ryanodine receptors	Use of radioactive labeling to screening for candidate 1,4-benzothiazepine compounds that bind to ryanodine receptor (RyR)
WO2002022122	NOVO NORDISK A/S	Use of compounds for the treatment of obesity	Inducing Ca ²⁺ release through RyR2 for the purpose of treating obesity.
US20070196856	Allergan, Inc.	Methods of determining activity of ryanodine receptor modulators	Methods for identifying modulators of RyR by observing whether a substance reduces the activity of a stimulated channel
US6780608	Boehringer Ingelheim International GmbH	Human type 3 ryanodine receptor protein and DNA molecules coding therefor	Therapies targeting dysfunctional RyR3