0031-6997/11/6303-700–727$25.00
PHARMACOLOGICAL REVIEWS
U.S. Government work not protected by U.S. copyright
*Pharmacol Rev* 63:700–727, 2011

Vol. 63, No. 3
3814/3690704
*Printed in U.S.A.*

ASSOCIATE EDITOR: DAVID R. SIBLEY

# Endoplasmic Reticulum $Ca^{2+}$ Handling in Excitable Cells in Health and Disease

Grace E. Stutzmann and Mark P. Mattson

*Department of Neuroscience, Rosalind Franklin University/The Chicago Medical School, North Chicago, Illinois (G.E.S.); and Laboratory of Neurosciences, National Institute on Aging Intramural Research Program, Baltimore, Maryland (M.P.M.)*

---

***Abstract*——The endoplasmic reticulum (ER) is a morphologically and functionally diverse organelle capable of integrating multiple extracellular and internal signals and generating adaptive cellular responses. It plays fundamental roles in protein synthesis and folding and in cellular responses to metabolic and proteotoxic stress. In addition, the ER stores and releases $Ca^{2+}$ in sophisticated scenarios that regulate a range of processes in excitable cells throughout the body, including muscle contraction and relaxation, endocrine regulation of metabolism, learning and memory, and cell death. One or more $Ca^{2+}$ ATPases and two types of ER membrane $Ca^{2+}$ channels (inositol trisphosphate and ryanodine receptors) are the major proteins involved in ER $Ca^{2+}$ uptake and release, respectively. There are also direct and indirect interactions**

Address correspondence to: Dr. Grace E. Stutzmann, Rosalind Franklin University/The Chicago Medical School, 3333 Green Bay Road, North Chicago, IL 60064. E-mail: grace.stutzmann@rosalindfranklin.edu

This article is available online at http://pharmrev.aspetjournals.org.
doi:10.1124/pr.110.003814.

**of ER $Ca^{2+}$ stores with plasma membrane and mitochondrial $Ca^{2+}$-regulating systems. Pharmacological agents that selectively modify ER $Ca^{2+}$ release or uptake have enabled studies that revealed many different physiological roles for ER $Ca^{2+}$ signaling. Several inherited diseases are caused by mutations in ER $Ca^{2+}$-regulating proteins, and perturbed ER $Ca^{2+}$ homeostasis is implicated in a range of acquired disorders. Preclinical investigations suggest a therapeutic potential for use of agents that target ER $Ca^{2+}$ handling systems of excitable cells in disorders ranging from cardiac arrhythmias and skeletal muscle myopathies to Alzheimer disease.**

## I. Introduction

### *A. Primer on Endoplasmic Reticulum Structure and Function*

The endoplasmic reticulum (ER[1]) is a membrane-bound organelle present in all eukaryotic cells, where it exhibits a range of structures, including tubules, vesicles, and complex net- or web-like formations (i.e., a reticulum). The ER membrane is believed to be initially generated as part of the nuclear envelop, which then expands and morphs into a complex reticulum that can extend for great distances within a cell (Petersen and Verkhratsky, 2007). Portions of the ER may then separate to form ER vesicles that can move to distant cellular compartments such as the long axons and dendrites of neurons (Aridor et al., 2004; Aridor and Fish, 2009). Two distinct types of ER are observed by electron microscopy: 1) rough ER is decorated by membrane-associated ribosomes and plays a major role in the synthesis of new proteins, and 2) smooth ER lacks ribosomes and is involved in lipid and steroid biosynthesis and $Ca^{2+}$ signaling (Shibata et al., 2006). The amount of each type of ER and their structural organization vary considerably among different types of cells. For example, smooth ER is abundant in adrenocortical cells that produce glucocorticoids (cortisol in humans and corticosterone in rodents) (Black et al., 2005). In contrast, endocrine secretory cells that produce and release large amounts of protein and peptide hormones possess large amounts of rough ER (Bendayan, 1989).

The structural organization of the ER is highly complex, in that it forms a reticulated network of tubules and cisternal regions that is widely distributed throughout the cytoplasm (Griffing, 2010). Tubules can transform into cisternae and vice versa; cisternae can generate tubules by forming tubules at their edges, and nodes and branches may shift to re-organize the ER network. Several proteins have been shown to control the generation and modification of ER structure. The formation of ER tubules requires reticulon protein Rtn4a/NogoA and DP1, whereas the fusion of different tubules is controlled by p47 and p97 proteins (Uchiyama and Kondo, 2005; Voeltz et al., 2006). In general, the smaller vesicular and tubular forms of smooth ER are highly mobile and can move within the cytoplasm in a purposeful manner. The movement of the ER toward the cell periphery is controlled by microtubules. The generation, maintenance, and remodeling of the ER is controlled by microtubule-associated proteins (kinesins and dyneins) and by tip attachment complexes located at the plus (growing) end of the microtubule (Bola and Allan, 2009). Actin filaments may also control ER movement, as demonstrated using an in vitro preparation in which it was shown that myosin on the ER membrane interacts with actin filaments to translocate ER vesicles in an ATP-dependent manner. Although the functional significance of intra-ER morphological changes and movement in cells is not well understood, it seems likely that such changes provide molecules produced in the ER (proteins, steroids, $Ca^{2+}$) to sites where they are needed.

ER structure and motility within subcellular compartments may be regulated by $Ca^{2+}$ signals. $Ca^{2+}$ is a major regulator of cytoskeletal dynamics in cells; $Ca^{2+}$ influx stimulates actin polymerization, and high levels of $Ca^{2+}$ cause microtubule depolymerization (Mattson, 1992). Such changes in microtubules and actin filaments will alter ER structure and motility as described above.

The ER often interacts with the plasma membrane, thereby serving an important role in the $Ca^{2+}$-mediated transduction of extracellular signals to the cell interior, including the nucleus. Much of this occurs through junctional units formed between integral membrane proteins involved in $Ca^{2+}$ homeostasis and adjacent channels in the ER. For example, stromal interaction molecule 1 (STIM1) is an ER transmembrane protein that interacts with proteins in the plasma membrane. STIM1 plays a pivotal role in store-operated $Ca^{2+}$ entry

---

[1] Abbreviations: 2-APB, 2-aminoethoxydiphenyl borate; 5-HT, 5-hydroxytrypamine; A$\beta$, amyloid $\beta$-peptide; AD, Alzheimer disease; ALS, amyotrophic lateral sclerosis; APP, amyloid precursor protein; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; Bcl-2, B-cell lymphoma 2; CALHM1, calcium homeostasis modulator 1; CaMK, $Ca^{2+}$/calmodulin-dependent protein kinase; CCD, central core disease; CICR, $Ca^{2+}$-induced $Ca^{2+}$ release; CPA, cyclopiazonic acid; CPVT, catecholaminergic polymorphic ventricular tachycardia; ER, endoplasmic reticulum; FAD, familial Alzheimer disease; GRP, glucose-regulated protein; Herp, homocysteine-inducible ER stress protein; $IP_3$, inositol triphosphate; $IP_3R$, inositol trisphosphate receptor; LTD, long-term depression; LTP, long-term potentiation; MmD, multi-minicore disease; MRS1845, *N*-propylargylnitrendipine; NF-$\kappa$B, nuclear factor-$\kappa$B; NMDA, *N*-methyl-D-aspartate; NMDAR, *N*-methyl-D-aspartate receptor; NPC, Niemann-Pick type C disease; PD, Parkinson disease; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PN, peripheral neuropathy; PS, presenilin; PUMA, p53–up-regulated modulator of apoptosis; RyR, ryanodine receptor; SA, sinoatrial; SERCA, sarcoplasmic-endoplasmic reticulum $Ca^{2+}$ ATPase; SKF96365, 1-(2-(3-(4-methoxyphenyl)-propoxy)-4-methoxyphenylethyl)-1*H*-imidazole; SOCE, store-operated calcium entry; SOD, superoxide dismutase; SR, sarcoplasmic reticulum; STIM, stromal interaction molecule; UPR, unfolded protein response; VAPB, vesicle-associated membrane protein-associated protein B; VGCC, voltage-gated $Ca^{2+}$ channel.

through associating with the plasma membrane channel, Orai; this important aspect is discussed in section II.B. In the context of ER structure and motility, it has also been reported that STIM1 binds directly to the microtubule-plus-end-tracking protein EB1 and mediates ER tubule growth via the microtubule tip attachment complex mechanism (Grigoriev et al., 2008). This may be the mechanism by which local $Ca^{2+}$ release from the ER, and/or influx through plasma membrane channels, increases (or decreases) the amount of ER associated with that particular region of the plasma membrane in which receptors that stimulate $Ca^{2+}$ influx are activated. It is noteworthy that STIM1-rich regions of the ER may preferentially interact with domains of the plasma membrane that are rich in sphingolipids and cholesterol, the so-called lipid rafts (Pani et al., 2008). Studies of neurons have shown that metabolism of sphingomyelin in lipid rafts modifies cell excitability and $Ca^{2+}$ influx through ligand-gated channels (Wheeler et al., 2009; Norman et al., 2010), suggesting a potential role for membrane lipids in controlling ER motility and $Ca^{2+}$ release.

Other examples of the ER forming junctions with the plasma membrane include the sodium/$Ca^{2+}$ exchanger NCX1, which forms $Ca^{2+}$ signaling complexes with SERCA2 and inositol trisphosphate ($IP_3$) receptor 1 ($IP_3R1$) by linkages through the cytoskeletal network (Lencesova et al., 2004). Interaction of the ER network with other organelles also allows for the intracellular transfer of $Ca^{2+}$. Mitochondria-ER communication has been well studied, because these organelles are highly abundant and subserve functions essential for cellular metabolism and survival. Physical links tether the outer mitochondrial membrane to the adjacent ER (Boncompagni and Protasi, 2007; Franzini-Armstrong, 2007), and the ER regulates mitochondrial energy metabolism through these close contacts by generating high $Ca^{2+}$ concentration microdomains that are a source for $Ca^{2+}$ uptake into the mitochondria via mitochondrial uniporters (Duchen, 1999). This source of $Ca^{2+}$ entry into the mitochondria has implications for cellular bioenergetics via $IP_3R$-mediated $Ca^{2+}$ release (Cárdenas et al., 2010) as well as serving neuroprotective functions (Eckenrode et al., 2010; Renvoisé and Blackstone, 2010).

## II. Endoplasmic Reticulum $Ca^{2+}$ Homeostasis and Signaling

In most cell types, including those discussed here, the ER is the largest intracellular organelle and extends throughout most cellular compartments. In addition to its role in storing, modifying, and transporting newly synthesized proteins, the ER is a high-capacity reservoir for intracellular $Ca^{2+}$, with intraluminal concentrations ranging from the high micromolar to low millimolar range (Berridge, 2002; Solovyova and Verkhratsky, 2002), roughly 4 to 5 orders of magnitude higher than the surrounding cytosol. This steep concentration gradient is the predominant driving force by which $Ca^{2+}$ exits the ER through one of several receptors/channels, such as the $IP_3$ receptor, ryanodine receptor (RyR), and leak channels. The ER can serve as a sink as well as a source for intracellular $Ca^{2+}$ signaling, transporting cytosolic $Ca^{2+}$ into the lumen through the sarcoplasmic-endoplasmic reticulum $Ca^{2+}$ ATPase (SERCA) pumps.

### *A. Endoplasmic Reticulum $Ca^{2+}$ Release Mechanisms*

*1. Inositol Trisphosphate Receptor.* There are two ER $Ca^{2+}$ channels that generate cell signaling-derived $Ca^{2+}$ release from the ER lumen to the cytosol. The first to be discussed is the $IP_3R$, which is an intracellular ligand-gated $Ca^{2+}$ channel, with six transmembrane domains in the carboxyl terminal, localized to the ER membrane (Bezprozvanny, 2005; Foskett et al., 2007; for review, see Yule et al., 2010). Its ligand, $IP_3$, is a second messenger generated from $G_q$-coupled or tyrosine kinase-linked receptors on the plasma membrane. These include, but are not limited to, the group I metabotropic glutamate receptors 1 and 5, 5-$HT_{2A}$ receptors, muscarinic acetylcholine receptors m1 and m3, $\alpha$1-adrenergic receptors, the $P2Y_1$ receptor, and several other types of P2Y and P2X receptors (James and Butt, 2002). Upon binding of the extracellular ligand to the receptor, phospholipase C is activated and hydrolyzes phosphatidylinositol bisphosphate into $IP_3$ and diacylglycerol; the former diffuses to the $IP_3R$ on the ER, and the latter activates protein kinase C (PKC). There are three mammalian subtypes of the $IP_3R$ (1, 2, and 3) with an overall sequence homology of 60 to 80%; however, the ligand binding domain, the $Ca^{2+}$-sensor domain, and the pore domains are highly conserved, and greater variability exists in the regulatory domains (Bezprozvanny, 2005; Foskett et al., 2007). This high sequence homology within binding and channel-forming domains is consistent with the experimental data demonstrating similar $IP_3$-binding, $Ca^{2+}$-gating, and ion conduction properties among the three $IP_3R$ subtypes, with more salient differences in their modulation. For example, with $Ba^{2+}$ (50 mM) as the charge carrier, the single-channel conductance for all subtypes is approximately 80 pS with a unitary current of ~1.9 pA. Affinity for $IP_3$ does seem to have subtle differences: $IP_3R2$ (0.10 $\mu$M) > $IP_3R1$ (0.27 $\mu$M) > $IP_3R3$ (0.40 $\mu$M) (Bezprozvanny, 2005; Tu et al., 2005). However, it should be noted that for most studies measuring unitary conductance properties (for $IP_3R$ and RyR), artificial membranes with nonphysiological ion concentrations were often used; therefore, properties in native cell membranes may be different.

$IP_3$ is not the only regulator of $IP_3R$ function; $Ca^{2+}$ itself is an allosteric modulator of the $IP_3R$ and plays a critical role in shaping the $IP_3R$-evoked $Ca^{2+}$ response. In general, this regulation follows a biphasic bell-shaped curve for all subtypes, such that low $Ca^{2+}$ concentrations (<300 nM) activate the channel and increase its open probability, whereas high $Ca^{2+}$ concentrations inhibit channel opening (Thrower et al., 2001; Foskett et al., 2007). This positive and negative feedback cycle is well suited for generating $Ca^{2+}$ oscillations or waves. The shapes of the biphasic curves are generally similar among the three $IP_3R$ subtypes, but minor differences may confer important functional differences. For example, the $Ca^{2+}$ activation of the $IP_3R1$ channels exhibits positive cooperativity, allowing for sharp and rapid increases in channel opening within a narrow $[Ca^{2+}]$. This dynamic would strongly support $Ca^{2+}$-induced $Ca^{2+}$ release (CICR), a process by which local elevations of intracellular $Ca^{2+}$ are amplified by $Ca^{2+}$ release from ER $Ca^{2+}$ stores. The open probability of the $IP_3R3$ channels increases over a broader range of $Ca^{2+}$ concentrations, with a higher affinity for $Ca^{2+}$, resulting in channel activity that is sensitive to low $[IP_3]$ (Mak and Foskett, 1997; Boehning et al., 2001; Tu et al., 2005; Foskett et al., 2007).

$Ca^{2+}$ cannot independently open the $IP_3R$ in the absence of $IP_3$; rather, it enhances the open probability of the $IP_3R$. This coordinates a scenario in which $Ca^{2+}$ released from one channel can facilitate release from the other, triggering a regenerative release of $Ca^{2+}$ within or between classes of $Ca^{2+}$ channels (Berridge, 1997). Because $Ca^{2+}$ signals can be encoded in both temporal and spatial domains, the oscillatory nature of $IP_3$-evoked $Ca^{2+}$ release can carry important functional significance. For example, $Ca^{2+}$ oscillations of specific frequencies can activate gene transcription or other signal transduction pathways (Li et al., 1998; Carrasco et al., 2004). The spatial spread and amplitude, or amount, of $Ca^{2+}$ release can trigger a variety of downstream $Ca^{2+}$-sensitive cascades depending upon relative binding affinities. In general, cytosolic $Ca^{2+}$ diffusion from the $IP_3R$ is rather limited, largely because of the strong $Ca^{2+}$ buffering capacity in the cytosol, and creates a steep concentration gradient originating from the ER release site. At the mouth of the channel, the $Ca^{2+}$ concentration can exceed >100 $\mu$M, whereas a few micrometers away, the concentration may be 1 $\mu$M, leaving a functional range of approximately 5 $\mu$M under normal conditions (Allbritton et al., 1992). However, under conditions of exaggerated ER $Ca^{2+}$ release, such as with certain Alzheimer disease-causing mutations or Huntington disease, these signaling patterns may be altered (Tang et al., 2005; Goussakov et al., 2010; Zhang et al., 2010).

The $IP_3R$ can operate as a homo- or heterotetramer, but the functional significance of the heteromeric forms is not well understood. Even further diversity among channel subtypes emerge with multiple alternative splice variants for each (Arredouani, 2004). The $IP_3R$ is ubiquitously expressed; the three subtypes have overlapping patterns of expression, and many cells express more than one form. Neurons are an exception, in that most express only the $IP_3R1$ subtype.

*2. Ryanodine Receptor.* The second ER $Ca^{2+}$ channel to be discussed is the RyR, a high-conductance relatively nonspecific cation channel (~100–150 pS for $Ca^{2+}$) in the SR/ER membrane. It is ubiquitously expressed in a large number of cells and supports a wide variety of $Ca^{2+}$ signaling events. It is similar in general structure to the $IP_3R$, particularly in the channel pore regions, yet at ~560 kDa, with numerous accessory proteins, the RyR is one of the largest channel complexes thus far identified (Mackrill, 2010). The mammalian genome includes three genes, located on different chromosomes, that encode the ryanodine receptor proteins RyR1, RyR2, and RyR3; these three RyRs exhibit approximately 70% sequence homology. Each isoform can be subject to post-translational and post-transcriptional regulation and can express numerous splice variants (Fill and Copello, 2002 for review). Despite the potential variability, individual channels function as a homotetramer.

The RyR1 is the most studied isoform to date and is predominant in skeletal muscle, where it functions in excitation-contraction coupling and muscle contraction. It has also been described in the Purkinje neurons of the cerebellum (Furuichi et al., 1994; Hertle and Yeckel, 2007). The RyR2 is heavily expressed in cardiac muscle and is also the predominant form found in the brain. The RyR3 follows more of a low level and widespread expression pattern and is found in striated, smooth, and cardiac muscle, as well as in T lymphocytes and in the brain—particularly regions involved in learning and memory (cortex and hippocampus) (Arredouani, 2004; Hertle and Yeckel, 2007).

The principal activator for all three RyR isoforms is $Ca^{2+}$ itself, generating the classic form of CICR. Other compounds can facilitate or modulate RyR-evoked $Ca^{2+}$ release, but the binding of $Ca^{2+}$ is a fundamental requirement for channel activation. The three isoforms display differing sensitivities to cytosolic $Ca^{2+}$ (RyR1 > RyR2 > RyR3) but have similar permeation properties characterized by large single-channel conductance values (>100 pS) (Fill and Copello, 2002). Like the $IP_3R1$, the RyR1 subtype has a biphasic, bell-shaped response curve with maximal release at ~5 $\mu$M and complete inhibition occurring in the low millimolar range. The RyR2 and RyR3 isoforms require substantially higher $Ca^{2+}$ concentrations (>10 mM) for inhibition, which is out of the physiological range for most cells (Fill and Copello, 2002). In addition, luminal $Ca^{2+}$ levels are thought to regulate the sensitivity of the RyR such that high luminal $Ca^{2+}$ levels increase its responsiveness to certain cytosolic agonists (Sitsapesan and Williams, 1997; Györke and Györke, 1998). The RyR can also be positively regulated by ATP and negatively by $Mg^{2+}$. As with the $IP_3Rs$, numerous signaling cascades can also impinge on RyR function, including the kinases cAMP-dependent protein kinase (PKA), PKC, cGMP-dependent protein kinase, and $Ca^{2+}$/calmodulin-dependent protein kinase II. Particularly relevant for the discussion on Alzheimer disease below (section VII), the RyR activity is also thought to be modulated by presenilin (PS), an ER-localized protease that cleaves a variety of type I membrane proteins (Rybalchenko et al., 2008; Zhang et al., 2010).

*3. Leak Channels.* The presence of the ER leak channel is inferred from the passive release of ER $Ca^{2+}$ upon blocking the counterbalancing SERCA pumps with compounds such as thapsigargin or cyclopiazonic acid (CPA). Although the leak channel has yet to be identified, several studies have proposed potential leak channel mechanisms. Flourakis et al. (2006) suggest that a passive $Ca^{2+}$ leak translocon-channel mediates the thapsigargin- and EGTA-induced $Ca^{2+}$ release and have shown that translocon-triggered $Ca^{2+}$ leak activates the store-operated $Ca^{2+}$ current. Pannexin 1, a newly discovered family of gap junction molecules, may also form $Ca^{2+}$-permeable leak channels in the ER membrane; however, this hypothesis requires further corroboration (Vanden Abeele et al., 2006). Two other potential $Ca^{2+}$ leak channels that may assist in maintaining ER $Ca^{2+}$ homeostasis are the translocon (Camello et al., 2002) and presenilin (Tu et al., 2006). Mutations in presenilin are linked to early-onset familial AD (FAD), and data suggest that the mutations impair an ER $Ca^{2+}$ leak function of presenilin (Tu et al., 2006; Nelson et al., 2007), resulting in increased ER $Ca^{2+}$ levels and increased vulnerability to degeneration (Guo et al., 1996, 1997, 1999).

### *B. Endoplasmic Reticulum $Ca^{2+}$ Uptake and Store Refilling*

*1. Sarcoplasmic-Endoplasmic Reticulum $Ca^{2+}$ ATPase Pumps.* Cytosolic $Ca^{2+}$ entry into the ER is mediated through the SERCA pump, a $Ca^{2+}$ ATPase that transfers $Ca^{2+}$ from the cytosol to the SR/ER lumen via ATP hydrolysis. In vertebrates, three distinct genes encode for the SERCA1, -2, and -3 proteins, and alternative splicing leads to a total of seven known isoforms (SERCA1a and -1b, SERCA2a and -2b, and SERCA3a, -3b, and -3c) (Andersen and Vilsen, 1998). SERCA1a is expressed exclusively in adult fast skeletal muscle fibers, whereas SERCA1b is expressed only in fetal muscle fibers. SERCA2a is expressed both in cardiac muscle and in the slow skeletal muscle fibers, whereas SERCA2b is ubiquitously expressed in nonmuscle tissues, particularly in the brain (Carafoli and Brini, 2000). SERCA3a, -3b, and -3c are variably expressed in various nonmuscle tissues but overlap with SERCA2b (Pacifico et al., 2003).

There are no significant functional differences observed between SERCA1a and -1b. However, SERCA1 pumps $Ca^{2+}$ twice as fast as SERCA2a, although their $Ca^{2+}$ affinities seem similar (Lytton et al., 1992; Sumbilla et al., 1999). The $Ca^{2+}$ affinity of SERCA2b ($K_m$, ~0.17 $\mu$M) is 2-fold higher than that of SERCA2a. Functional studies of SERCA3 indicate it has a lower $Ca^{2+}$ affinity ($K_m$, ~2 $\mu$M), a high optimal pH (7.2–7.4 versus 6.8–7.0) and 10-fold higher sensitivity to inhibition. The affinity for ATP is similar for all SERCA isoforms (0.02–0.05 $\mu$M). The particular biochemical characteristics and the restricted tissue distribution of SERCA3 might suggest a role in specialized signaling functions. In terms of complementary roles within cells, ablation studies indicate that removal of one SERCA isoform often does not impair primary cellular function, suggesting distinct roles of different SERCA pumps for $Ca^{2+}$ homeostasis (Dode et al., 1992; Arredouani, 2004).

*2. Store-Operated Calcium Entry.* Beyond SERCA pumps, there is much interest in the complex detection system by which the ER signals the $Ca^{2+}$ store-refilling processes, and, until recently, this mechanism had eluded scientists for more than 20 years. In the past, ER $Ca^{2+}$ depletion had been observed in many cell types to result in a $Ca^{2+}$ current through the plasma membrane, which served to refill the ER stores (Cahalan, 2009), but the mystery lay in determining how an intracellular organelle signaled the plasma membrane to trigger $Ca^{2+}$ entry and funnel it specifically to the ER. After extensive RNA interference screening, as well as cellular, molecular, and physiological analysis, two critical protein families were determined to be necessary and sufficient for the function of store-operated $Ca^{2+}$ entry: STIM (stromal interacting molecule) and Orai. The discovery of STIM, and STIM1 function in particular, transformed the highly debated store-operated hypothesis into a validated mechanism. STIM1 is a type I membrane protein localized in the ER and, with an unpaired $Ca^{2+}$-binding EF hand, serves as a luminal $Ca^{2+}$ sensor. Orai is a plasma membrane protein with four transmembrane domains and functions as the highly selective $Ca^{2+}$ channel that is gated through interactions with STIM (Hewavitharana et al., 2007). An in-depth review of the molecular basis of store-operated $Ca^{2+}$ entry (SOCE) was recently published (Smyth et al., 2010). A summary description of SOCE is as follows. When ER $Ca^{2+}$ stores are filled sufficiently, $Ca^{2+}$ binding to EF hands keeps STIM distributed in the ER membrane and distanced from the plasma membrane. However, upon depletion of ER stores and disassociation of $Ca^{2+}$ from the EF hands, Stim1 will reassemble within the ER membrane and oligomerize at sites immediately adjacent to the plasma membrane. In this conformation, STIM1 binds with SOCE channels of the Orai family in the plasma membrane. The Stim1-Orai complex stimulates store-activated $Ca^{2+}$ influx, thereby replenishing ER stores with $Ca^{2+}$ funneled from the extracellular space directly into the ER (Lewis, 2007; Prakriya, 2009). It is noteworthy that the proximal step of STIM1 oligomerization is the key triggering event by which $Ca^{2+}$ store depletion controls SOCE (Luik et al., 2008; Lee et al., 2010).

Additional mechanisms involved in SOCE have been suggested. These include post-translational modifications of STIM1 levels, cellular localization, and/or interaction with Orai proteins. Phosphorylation of STIM1 on Ser486 and Ser668 was found to inhibit the movement of STIM1 to plasma membrane foci and thereby to inhibit SOCE (Smyth et al., 2009). Studies of cultured hippocampal neurons suggest that STIM1 is ubiquitinated and that proteasome inhibition increases the amount of plasma membrane-associated STIM1 when ER stores are depleted (Keil et al., 2010). Moreover, overexpression of the E3 ubiquitin ligase PLOSH (plenty of SH3s) reduces STIM1 surface levels, suggesting that ubiquitination may play a role in SOCE (Keil et al., 2010), a process potentially involved in modification of SOCE under conditions of proteotoxic stress. Finally, although Orai proteins are the most established SOCE channels, STIM1 has also been reported to activate transient receptor potential C1 channels in ER-plasma membrane microdomains (Pani et al., 2009).

### *C. Regulation of $Ca^{2+}$ within the Endoplasmic Reticulum*

Much of the $Ca^{2+}$ in the ER is in a free, unbuffered state; although the total store content may exceed 1 mM in some cells, estimates of free $[Ca^{2+}]$ range from 100 to 800 $\mu$M (Bygrave and Benedetti, 1996; Alvarez and Montero, 2002; Solovyova and Verkhratsky, 2002). This allows for rapid diffusion of $Ca^{2+}$ throughout the lumen (faster than through the cytosol) and, therefore, throughout most compartments of the cell (Park et al., 2008). Still, high-capacity $Ca^{2+}$ buffers play an important role in maintaining ER homeostasis. Calreticulin is the most abundant buffering protein and contains 20 to 50 low-affinity ($K_d$, ~1 mM) $Ca^{2+}$ binding sites. This particular buffer is unique in that it also serves as a chaperone protein and regulator/$[Ca^{2+}]$ sensor for SERCA function by binding to and activating SERCA pump activity once $Ca^{2+}$ levels fall below threshold levels (Verkhratsky, 2005). Calsequestrin, which is predominant in skeletal muscle cells, is another high-capacity and low-affinity $Ca^{2+}$ buffer with binding properties similar to those of calreticulin. In addition, glucose-regulated protein (GRP) 94, GRP78 (also known as BiP), and the CREC family of proteins, which are multiple EF-hand proteins including reticulocalbin, 55-kDa ER $Ca^{2+}$-binding protein, reticulocalbin-3, 45-kDa $Ca^{2+}$-binding protein, and calumenin, can also be found as low-affinity $Ca^{2+}$ buffering proteins in the ER (Verkhratsky, 2005).

The $Ca^{2+}$ concentration within the ER lumen also regulates the opening of both $IP_3$ receptors and RyR (Burdakov et al., 2005). Early studies in permeabilized hepatocytes provided evidence that an increase of intraluminal $Ca^{2+}$ levels increased the sensitivity of $IP_3$ receptors to $IP_3$ (Nunn and Taylor, 1992). Although subsequent studies confirmed a positive effect of intraluminal $Ca^{2+}$ on $IP_3$ receptors (Parys et al., 1993), the molecular mechanism by which $Ca^{2+}$ affects $IP_3$ receptor channel activity is unknown. In contrast to $IP_3$ receptors, the regulation of RyR by intraluminal $Ca^{2+}$ is well established and understood, in part. The open probability of RyR, and their sensitivity to caffeine and cytosolic $Ca^{2+}$, are directly affected by intraluminal $Ca^{2+}$ levels. It has been shown in studies of skeletal muscle and cardiac cells that the ER $Ca^{2+}$ release is increased by as much as 20-fold by a 10-fold increase in the intraluminal ER $Ca^{2+}$ concentration (Donoso et al., 1995).

Recordings of single RyR channels of native RyRs in SR vesicles in the presence of Mg-ATP using $Cs^+$ as the charge carrier showed that raising luminal $Ca^{2+}$ concentration from 20 $\mu$M to 5 mM increased the open channel probability (Györke et al., 2004). By performing the recordings in the presence or absence of calsequestrin, triadin 1 and junctin provided evidence that these three proteins confer RyR luminal $Ca^{2+}$ sensitivity. These data suggest that calsequestrin serves as a luminal $Ca^{2+}$ sensor that inhibits the channel at low luminal $Ca^{2+}$ levels, whereas triadin 1 and/or junctin may be required to mediate interactions of calsequestrin with RyR. In cultured pheochromocytoma cells and dorsal root ganglion neurons, an increase in ER $Ca^{2+}$ resulted in increased sensitivity of $Ca^{2+}$ release to caffeine (Shmigol et al., 1996; Koizumi et al., 1999).

Whereas changes in the cytosolic $Ca^{2+}$ concentration within a physiological range do not have a major effect on SERCA activity, ER luminal $Ca^{2+}$ plays a major role in regulating SERCA activity. In studies in which ER $Ca^{2+}$ levels were directly compared with ER $Ca^{2+}$ uptake velocity, a reduction of ER $Ca^{2+}$ levels was found to result in an increased velocity of SERCA-mediated $Ca^{2+}$ uptake (Mogami et al., 1998). The physiological importance of ER $Ca^{2+}$ store depletion in activation of SERCA has been established in studies of cultured pancreatic acinar cells and neurons. Induced ER $Ca^{2+}$ depletion resulted in a large 5- to 8-fold increase in the velocity of ER $Ca^{2+}$ uptake (Mogami et al., 1998; Solovyova et al., 2002b). In the latter study, an additional experiment in which the cytosolic $Ca^{2+}$ concentration was held constant demonstrated that the relationship between the ER $Ca^{2+}$ concentration and the ER $Ca^{2+}$ uptake velocity was independent of a change of cytosol $Ca^{2+}$ levels.

### *D. Protein Translation and Quality Control*

The ER is a protein synthesis factory and sensor of cellular stress (Naidoo, 2009). All integral membrane proteins and all secreted proteins are folded and post-translationally modified (primarily glycosylation) in the ER. Because many different proteins are being synthesized, folded, and glycosylated simultaneously, the concentration of proteins in the ER is much greater than elsewhere in the cell, possibly as high as 100 mg/ml (Stevens and Argon, 1999). To prevent the aggregation of newly generated proteins, the ER contains an array of protein chaperones, foldases, and carbohydrate-processing enzymes. The folding of proteins begins during the translation process as the protein traverses the ER membrane through the translocon protein complex. Post-translational folding occurs within the ER lumen and involves the participation of protein chaperones and protein folding sensors that include GRP78 (also known as BiP), GRP94, calnexin, calreticulin, and protein sulfide isomerase. GRP78, a member of the 70-kDa heat-shock protein family, interacts with newly synthesized proteins as they pass through the translocon. GRP78 interacts with hydrophobic domains of proteins by an ATP-dependent process and thereby aids proper folding of the proteins. This critical chaperone function of GRP78 is therefore vulnerable to cellular energy depletion, which therefore results in the abnormal accumulation of unfolded proteins in the ER. GRP78 is a master regulator of the unfolded protein response (UPR), which is described later in this section.

Three major ER protein chaperones are $Ca^{2+}$-binding proteins. GRP94 is an abundant ER protein chaperone of the 90-kDa heat-shock protein family that binds up to 15 $Ca^{2+}$ ions. GRP94 binds to proteins after they have been released from GRP78 but before they are completely assembled, in contrast to GRP78, which binds most if not all nascent proteins, GRP94 interacts with a limited number of proteins. Calreticulin and calnexin are $Ca^{2+}$-binding lectin proteins that play a major role in the quality control of glycated proteins in the ER; calreticulin is located in the ER lumen, and calnexin is a transmembrane protein. After N-linked oligosaccharides are added to proteins in the ER, enzymes trim the carbohydrate chains in a process that is tightly controlled by calreticulin and calnexin. In addition to its chaperone function, calreticulin plays important roles in the regulation of intracellular $Ca^{2+}$ homoeostasis and ER $Ca^{2+}$ pool size (Michalak et al., 2009). As described above, calreticulin negatively regulates SOCE. Calreticulin deficiency results in impaired agonist-induced $Ca^{2+}$ release, reduced ER $Ca^{2+}$ store capacity, and decreased concentration of free $Ca^{2+}$ in the ER lumen. Calnexin may regulate ER $Ca^{2+}$ homoeostasis by interacting with SERCA proteins. When calnexin was coexpressed with SERCA2b in frog oocytes, intracellular $Ca^{2+}$ oscillations were inhibited (Roderick et al., 2000). C-terminal amino acids of calnexin are essential for its interaction with SERCA2b, and $IP_3$-mediated $Ca^{2+}$ release results in dephosphorylation of Ser562, which then reduces the interaction of calnexin and SERCA2b.

### *E. Endoplasmic Reticulum Stress, $Ca^{2+}$, and Cell Death*

The UPR is a programmed sequence of events that in the first instance protects cells against death under conditions of metabolic, ionic, and protopathic stress. When too many proteins are not being properly folded and post-translationally modified in the ER, GRP78 orchestrates multiple processes that result in the halting of translation of most proteins other than those necessary for maintenance of cell viability. The UPR can be triggered by glucose/energy deprivation, alterations in $Ca^{2+}$ homeostasis, oxidative stress, and ischemia. Three key events of the UPR are as follows: a rapid increase in the expression of GRP78; activation of protein kinase RNA-like endoplasmic reticulum kinase, which inhibits protein translation by phosphorylating the eukaryotic initiation factor-2$\alpha$; and proteasomal degradation of misfolded proteins via ER-associated degradation. In addition, the transcription factor NF-$\kappa$B may be activated by ER stress, resulting in the up-regulation of proteins that promote cell survival, including Mn-SOD and Bcl-2 (Mattson and Meffert, 2006). Together, these events protect excitable cells by reducing ER and mitochondrial stress.

Cell death can be and often is triggered by ER $Ca^{2+}$ release in both physiological and pathological settings (for review, see Pinton et al., 2008; Camandola and Mattson, 2011). Blockade of SERCAs with thapsigargin is sufficient to initiate the death of many types of excitable cells, including neurons (Guo et al., 1997), cardiac myocytes (Nickson et al., 2007), and pancreatic $\beta$ cells (Luciani et al., 2009). In pancreatic $\beta$ cells, thapsigargin induces apoptosis that involves phosphorylation of protein kinase RNA-like endoplasmic reticulum kinase and eukaryotic initiation factor-2$\alpha$ and activation of the classic mitochondria-mediated caspase 3-dependent apoptosis pathway (Luciani et al., 2009). Thapsigargin-induced apoptosis was mediated by $Ca^{2+}$ release through $IP_3$ receptor and RyR channels. A key event in mitochondria-mediated apoptosis in excitable cells (and nonexcitable cells as well) is the opening of membrane permeability transition pores and the release of cytochrome *c* (for review, see Mattson and Kroemer, 2003). Proteins of the Bcl-2 family control the permeability of the mitochondrial membrane; some members of this protein family stabilize the mitochondrial membrane [e.g., Bcl-2 and B-cell lymphoma-extra large (Bcl-xL)], whereas others induce opening of the permeability transition pores [Bcl-2–associated X protein (Bax), Bcl-2-associated death promoter (Bad), and p53–up-regulated modulator of apoptosis (PUMA)]. For example, PUMA is a pro-apoptotic Bcl-2 family member that is rapidly up-regulated in cardiac myocytes in response to ER stress induced by either thapsigargin or tunicamycin (Nickson et al., 2007). Depletion of PUMA from cardiac myocytes using molecular genetic methods rendered the cells resistant to being killed by ER stress.

Increasing evidence suggests that Bcl-2 proteins also interact with the ER membrane, where they may modify $Ca^{2+}$ release and control cross-talk between the ER and mitochondria (Lam et al., 1994; Rodriguez et al., 2011). It is noteworthy that a $Ca^{2+}$-mediated mitochondria–ER positive feedback pathway has been described that likely plays a role in hastening cell death once the apoptotic process is triggered. In the latter mechanism, the cytochrome *c* released from mitochondria binds to $IP_3$ receptors and thereby promotes $Ca^{2+}$ release, which, in turn, acts on mitochondria to enhance opening of permeability transition pores (Boehning et al., 2003).

## III. Pharmacology of Endoplasmic Reticulum $Ca^{2+}$-Handling Systems

Agents that selectively activate or inhibit $Ca^{2+}$ release from the ER range from the most widely used "drug" to exotic chemicals isolated from marine organisms. Caffeine (Fig. 1) is a chemical present in relatively high amounts in coffee and tea and is an additive to many soft drinks. It increases alertness and can improve performance in mental and physical tasks but can also have undesirable side effects, including dehydration, increased heart rate, and anxiety (Lara, 2010). Caffeine activates ryanodine receptors resulting in $Ca^{2+}$ release from the ER, which is a mechanism by which caffeine affects the excitability of neurons, cardiac myocytes, and skeletal muscle cells (Butanda-Ochoa et al., 2006). In addition to activating RyR, caffeine is an effective inhibitor of $IP_3$ receptors (Toescu et al., 1992), an action that increases its ability to promote the selective release of $Ca^{2+}$ from ryanodine-sensitive stores. Several endogenous bioactive molecules with structures similar to that of caffeine have been shown to increase the opening of RyR, including adenosine, inosine, xanthine, and uric acid (Butanda-Ochoa et al., 2006).

Agents other than caffeine that activate ryanodine receptors have been reported to exhibit therapeutic benefits in animal models of several different disorders. For example, in a rodent model of stroke in which there is unilateral damage to the sensorimotor cortex, treatment with inosine improved functional recovery by a mechanism that involved induction of genes encoding proteins involved in axon growth (Zai et al., 2009). The xanthine derivative propentofylline was reported to be effective in preserving cognitive function in patients with Alzheimer disease (Kittner et al., 1997), although whether its efficacy is the result of actions on ryanodine receptors, phosphodiesterases, or another mechanism is unknown. It is noteworthy that recent findings suggest that individuals with relatively higher plasma uric acid levels are at reduced risk of developing Alzheimer disease (Irizarry et al., 2009), and uric acid protected cultured neurons from being killed by amyloid $\beta$-peptide (Guo et al., 1999). Uric acid analogs with increased solubility were reported to protect the brain against ischemic injury (Haberman et al., 2007) and to accelerate cutaneous wound healing (Chigurupati et al., 2010). However, the relative contributions of the inherent antioxidant activity of uric acid, versus its potential actions on ryanodine receptors, to the beneficial effects of uric acid in these experimental models remains to be determined.

Although activation of RyR can improve the functionality of some cell types, including neurons, there are several diseases in which blocking RyR-mediated $Ca^{2+}$ release is desirable. Malignant hyperthermia is a life-threatening inherited disorder most often caused by mutations in the gene encoding RyR1. Patients may exhibit no abnormalities until they are subjected to volatile anesthetics (halothane, isoflurane, and others) for surgery; the anesthetic triggers a rapid excessive opening of RyR1, resulting in muscle contraction and increased body temperature. Treatment of patients with the RyR

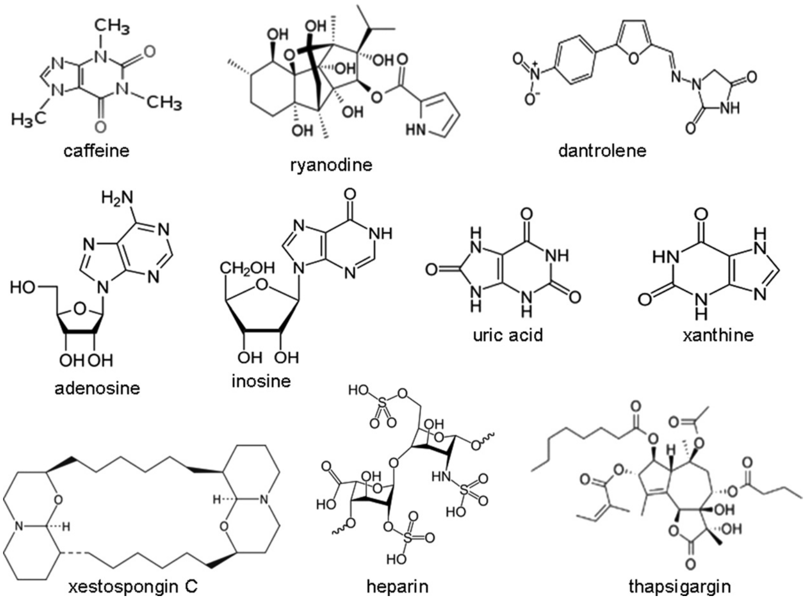

FIG. 1. Structures of agents that activate or inhibit ryanodine receptors, $IP_3$ receptors, or the ER $Ca^{2+}$-ATPase. Caffeine activates ryanodine receptors, ryanodine activates (low concentrations) or inhibits (high concentrations) ryanodine receptors, and dantrolene inhibits ryanodine receptors. Adenosine, inosine, uric acid, and xanthine have all been reported to modulate ryanodine-sensitive ER $Ca^{2+}$ stores. Xestospongin C and low-molecular-weight heparin inhibit $IP_3$ receptor-mediated $Ca^{2+}$ release. Thapsigargin selectively inhibits the ER $Ca^{2+}$-ATPase.

inhibitor dantrolene (Fig. 1) can greatly reduce mortality and morbidity (Rosenberg et al., 2007). Another type of disorder in which dantrolene is often used is spasticity, in which muscles contract uncontrollably (Young, 1987). Ischemia-reperfusion damage to the heart (myocardial infarction) and brain (stroke) is a common cause of morbidity and mortality. The ischemic damage involves cellular $Ca^{2+}$ overload, and dantrolene treatment can reduce cellular damage and cell death and can improve functional outcome in animal models of myocardial infarction, stroke, and ischemia (Wei and Perry, 1996; Nakayama et al., 2002; Muehlschlegel and Sims, 2009; Boys et al., 2010). Preclinical studies also showed that dantrolene can protect neurons against damage caused by amyloid β-peptide in an experimental in vitro model relevant to Alzheimer disease (Guo et al., 1997). However, in vivo studies indicate that long-term dantrolene feeding resulted in increased amyloid load, loss of synaptic markers, and increased neuronal atrophy in an aged AD mouse model (Zhang et al., 2010).

As mentioned above, there are three types of $IP_3Rs$ that function as $Ca^{2+}$ release channels in the ER. $IP_3Rs$ are phosphorylated by several major kinases, including PKA, cGMP-dependent protein kinase, and $Ca^{2+}$/calmodulin-dependent protein kinase (CaMK), that can modulate its sensitivity to $Ca^{2+}$ and $IP_3$. $IP_3R$ proteins have been shown to interact with several proteins involved in cellular signal transduction, including calmodulin, Homer, huntingtin-associated protein-1A, receptor of activated protein kinase C 1, protein phosphatase-2A, and ankyrin (Mikoshiba, 2007). It is noteworthy that $IP_3R$ in the ER membrane may also interact with the plasma membrane $Na^+/K^+$-ATPase to regulate cellular excitability and $Ca^{2+}$ oscillations (Miyakawa-Naito et al., 2003). Although numerous cell surface receptors are coupled to the GTP-binding protein $G_{q11}$, phospholipase C activation, and generation of $IP_3$ (Putney, 1987), surprisingly few low-molecular-weight agonists or antagonists of $IP_3R$ have been identified. Because $IP_3$ is hydrophilic and so does not readily cross membranes, membrane-permeant analogs of $IP_3$ have been developed and used in cell culture systems to elucidate the effects of $IP_3$ generation (in the absence of diacylglycerol production) on cell behaviors. For example, treatment of cultured astrocytes with a membrane-permeant analog of $IP_3$ protected them from being damaged by oxidative stress, suggesting a role for ER $Ca^{2+}$ release in the up-regulation of cytoprotective pathways (Wu et al., 2007).

One naturally occurring chemical inhibitor of $IP_3R$ is xestospongin C (Fig. 1), which was first isolated from Pacific basin sponges and has been shown to have vasodilatory properties (Nakagawa and Endo, 1984). More than a decade later, xestospongin C was shown to be a selective blocker of $IP_3R$ (Gafni et al., 1997). By inhibiting $IP_3$-mediated ER $Ca^{2+}$ release, xestospongin C has a range of biological activities on various cell types, including suppressing antigen-induced degranulation of mast cells (Oka et al., 2002), blocking $IP_3R$-mediated hypoxic preconditioning in hippocampal neurons (Bickler et al., 2009), and protecting neurons against the cell death-promoting action of a mutant form of presenilin-1 that causes early-onset inherited Alzheimer disease (Mattson et al., 2000). Xestospongin C also blocked the adverse effect of a presenilin-1 mutation in rendering neurons vulnerable to being damaged by the volatile anesthetic isoflurane (Liang et al., 2008). However, additional actions of xestospongin C on ER $Ca^{2+}$ handling have been reported, including inhibition of SERCA pumps and depletion of $Ca^{2+}$ stores without inhibiting $IP_3$-induced $Ca^{2+}$ release in sensory neurons (Solovyova et al., 2002a).

Another antagonist at $IP_3R$ that has been widely used to elucidate the involvement of $Ca^{2+}$ release from $IP_3$-sensitive ER stores in experimental models is 2-aminoethoxydiphenyl borate (2-APB). For example, 2-APB was used to establish a role for $IP_3$-induced $Ca^{2+}$ release in the generation of the entire physiological response of photoreceptors to light in the horseshoe crab (Fein, 2003); to show that $IP_3R$ are essential for the propagation of $Ca^{2+}$ oscillations in response to depolarization in sensory neurons (Zeng et al., 2008); and to demonstrate a pivotal role for $IP_3R$-mediated $Ca^{2+}$ release in the vasoconstriction of small arteries (Snetkov et al., 2003). However, although 2-APB has been widely used to evaluate the involvement of $IP_3$ receptors in the generation of $Ca^{2+}$ signals, it is not a very specific agent. Indeed, 2-APB has been shown to exert a greater inhibitory effect on SOCE than on $Ca^{2+}$ release (Bootman et al., 2002). In addition to xestospongins and 2-APB, low-molecular-weight heparin has also been demonstrated to be a competitive antagonist of the $IP_3$ receptor (Wu et al., 1994), although its use in this capacity has been mostly limited to cell culture and in vitro studies.

Although the bulk of the data using $IP_3R$ antagonists has come from studies of cultured cells, a few studies have demonstrated the ability of such agents to modify physiological and pathological processes in vivo. For example, treatment of chicks with xestospongin C impairs the formation of long-term memory (Baker et al., 2008), and treatment of worms (*Caenorhabditis elegans*) with xestospongin C phenocopies $IP_3R$ mutant worms that exhibit defects in the migration of epithelial cells during development (Thomas-Virnig et al., 2004). Another study reported that intracerebroventricular administration of low-molecular-weight heparin reversed tolerance to morphine in mice (Smith et al., 1999), suggesting a role for $IP_3R$-mediated $Ca^{2+}$ release in morphine tolerance. Additional studies also suggest that heparin may offer neuroprotection in Alzheimer disease, Huntington disease, and stroke (Mary et al., 2001; Bergamaschini et al., 2004; Tang et al., 2005). In a rat model of ischemia-reperfusion injury to the liver, administration of 2-APB protected liver cells from being damaged, and this was associated with reduced accumulation of $Ca^{2+}$ in mitochondria of the liver cells (Nicoud et al., 2007). In another study, 2-APB treatment protected striatal neurons against neurodegeneration caused by mutant huntingtin protein in a mouse model of Huntington disease (Tang et al., 2005).

Thapsigargin (a guaianolide compound of plant origin) and CPA are highly selective inhibitors of the SERCA pump, without effects on the plasma membrane $Ca^{2+}$ ATPase (Fig. 1). As a result, $Ca^{2+}$ is released from the ER in amounts that depend upon the concentration of thapsigargin and CPA; high concentrations of these agents are often used to completely deplete the ER of $Ca^{2+}$ (Darby et al., 1993). Thapsigargin is an irreversible inhibitor of SERCAs with a $K_d$ of 20 nM. Although inhibition of SERCA is the most prominent mechanism by which thapsigargin affects cellular $Ca^{2+}$ homeostasis, it can also inhibit voltage-gated $Ca^{2+}$ channels (VGCCs) and $Na^+$ channels. Thapsigargin and CPA have been widely used to elucidate the roles of ER $Ca^{2+}$ stores in a range of physiological processes. Indeed, more than 7000 publications in which thapsigargin was employed are listed on PubMed, and approximately 600 studies that used CPA. Treatment of cultured cells with thapsigargin or CPA in the absence of extracellular $Ca^{2+}$ results in a transient elevation of the cytosolic $Ca^{2+}$ concentration as the ER $Ca^{2+}$ pool is depleted. In the presence of extracellular $Ca^{2+}$, treatment of cells with thapsigargin or CPA induces a larger and sustained elevation of cytosolic $Ca^{2+}$ levels as the result of $Ca^{2+}$ influx through plasma membrane channels (Fig. 2A). The influence of various genetic and environmental factors on the amount of $Ca^{2+}$ stored in the ER can be determined by comparing the amounts of $Ca^{2+}$ released from the ER in response to thapsigargin or CPA (in the absence of extracellular $Ca^{2+}$) in cells expressing different genes of interest or maintained under different environmental conditions. For example, thapsigargin was used to establish that mutant forms of presenilin-1 that cause Alzheimer disease result in an increased pool of ER $Ca^{2+}$ (Guo et al., 1996) (Fig. 2B).

From the perspective of developing therapeutic agents that target ER $Ca^{2+}$-handling proteins, several major hurdles must be crossed. Small-molecular-weight agents that selectively inhibit or activate different ryanodine or $IP_3$ receptor subtypes should be developed. This might be accomplished by using high-throughput assays to screen libraries and/or by synthesizing analogs of existing ER $Ca^{2+}$-modulating agents ($IP_3$, ryanodine, caffeine, dantrolene, thapsigargin, xestospongins, etc.). A useful small molecule should be lipophilic so that it can pass through the plasma membrane and reach its molecular target in the ER; this property may also permit the agent to enter the central nervous system. Alternatively, known agonists or antagonists of $IP_3R$, ryanodine receptors, or SERCAs might be coupled to carrier molecules. One clever approach toward targeting ER $Ca^{2+}$-modulating drugs to specific cell types has been reported in which thapsigargin is coupled to a targeting peptide such that this "prodrug" is inactive and is then activated by the prostate cancer-specific protease prostate-specific antigen. The prostate-specific antigen-activated thapsigargin prodrug has been shown to be selectively toxic to prostate cancer cells in vivo (Denmeade and Isaacs, 2005).

## IV. Endoplasmic Reticulum $Ca^{2+}$ within Specific Cells and Systems

### *A. Cardiac Cells*

The heart beats continuously throughout life, generating the rhythmic pumping force that propels the blood

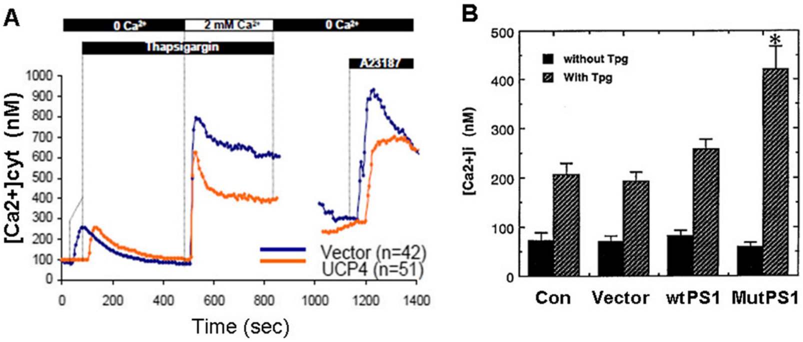

FIG. 2. Examples of pharmacological and genetic manipulations of ER $Ca^{2+}$ dynamics. A, cultured neural cells were transfected with an empty control vector or with an expression vector containing the cDNA encoding the mitochondrial uncoupling protein 4 (UCP4). Intracellular $Ca^{2+}$ concentrations were then monitored in the cells by ratiometric imaging of the $Ca^{2+}$ indicator dye fura-2 at baseline and during exposures to the indicated experimental treatments. 0 $Ca^{2+}$, culture medium lacking $Ca^{2+}$; 2 mM $Ca^{2+}$, culture medium containing 2 mM $Ca^{2+}$; thapsigargin (1 $\mu$M); and A23187, the $Ca^{2+}$ ionophore A23187 (calcimycin; 10 $\mu$M). Note that $Ca^{2+}$-induced $Ca^{2+}$ influx (in response to addition of extracellular $Ca^{2+}$ in the presence of thapsigargin is attenuated in cells overexpressing UCP4. See Chan et al. (2006) for additional information. B, neural cells expressing a presenilin-1 mutation that causes Alzheimer disease exhibit an elevated ER pool of $Ca^{2+}$. The indicated clones of PC12 cells were exposed to vehicle or 1 mM thapsigargin and the intracellular $Ca^{2+}$ concentration was measured 30 min later. Con, untransfected control cells; vector, cells transfected with empty vector; wtPS1, cells overexpressing wild type presenilin-1; mutPS1, cells overexpressing the L286V presenilin-1 missense mutation. Note that thapsigargin-induced elevation of the intracellular $Ca^{2+}$ level was greater in cells expressing mutant presenilin-1 compared with each of the other three cell clones. [Modified from Guo Q, Furukawa K, Sopher BL, Pham DG, Xie J, Robinson N, Martin GM, and Mattson MP (1996) Alzheimer's PS-1 mutation perturbs calcium homeostasis and sensitizes PC12 cells to death induced by amyloid beta-peptide. *Neuroreport* **8**:379–383. Copyright © 1996 Lippincott Williams & Wilkins. Used with permission.]

through the entire circulatory system to provide every cell in the body with nutrients and various signals that allow them to respond adaptively to environmental demands. The role of ER $Ca^{2+}$ handling in the regulation of heart rate, myocardial contraction, blood pressure, and blood flow has been the subject of considerable investigation. In this section, we review some of the findings concerning characteristics of ER $Ca^{2+}$ dynamics in several different cell types involved in the processes described in the preceding sentence with a focus on sinoatrial node (pacemaker) cells and ventricular myocytes. Evidence that perturbed ER $Ca^{2+}$ regulation contributes to the pathogenesis of cardiovascular diseases will then be described.

Pacemaker cells in the sinoatrial (SA) node exhibit oscillations of cytosolic $Ca^{2+}$ levels that seem to underlie the rhythmicity of the resting heart beat; these $Ca^{2+}$ oscillations are controlled by both plasma membrane ion channels and $Ca^{2+}$ release from the ER (Mangoni and Nargeot, 2008). The exact details of how pacemaker cells generate, maintain, and modulate the $Ca^{2+}$ oscillations are not fully understood. However, multiple ER $Ca^{2+}$-handling proteins are central to this process. Early studies demonstrated the requirement of $Ca^{2+}$ release through ryanodine receptors in the regulation of SA node automaticity (Hata et al., 1996). In addition, it is well established that the RyR agonist caffeine can increase heart rate and that this occurs, at least in part, by caffeine's agonistic action on the RyR (Dietrich et al., 1976). Other studies have suggested that the $Ca^{2+}$-pumping kinetics of the SERCA regulates the timing of ER $Ca^{2+}$ release in SA node cells (Vinogradova et al., 2010). With regard to plasma membrane $Ca^{2+}$-handling systems, it has been suggested that the $Na^+/Ca^{2+}$ exchanger is a critical component of the intrinsic SA node cellular clock (Bogdanov et al., 2001). Changing the tachometer setting of the SA node clock is controlled largely by activity of sympathetic nerves that innervate these cells; the norepinephrine released from sympathetic nerve terminals activates $\beta$-adrenergic receptors resulting in an increase in beats per minute of the heart.

The development of the cardiovascular system is critically dependent on ER $Ca^{2+}$ release. Cardiomyocytes differentiated from embryonic stem cells lacking RyR2 exhibit impaired development of spontaneous rhythmic contractions, which is associated with an absence of ER $Ca^{2+}$ sparks (Yang et al., 2002). Mice lacking both $IP_3R1$ and $IP_3R2$ (but not mice lacking either $IP_3R$ alone) die during embryonic development at 11.5 days of gestation; the embryos exhibit severe defects of the ventricular myocardium and the atrioventricular canal of the heart (Uchida et al., 2010). The plasma and ER membranes that house proteins involved in excitation-contraction coupling are closely apposed to each other in cardiac myocytes in structures called *t*-tubules. In addition to voltage-dependent $Ca^{2+}$ channels in the plasma membrane and RyR and IP3R in the ER, *t*-tubules are enriched in ion-motive ATPases and $Na^+/Ca^{2+}$ exchange proteins, presumably to allow rapid restoration of cytosolic $Ca^{2+}$ levels after excitation-induced $Ca^{2+}$ influx and release (Orchard and Brette, 2008). In ventricular myocytes, $Ca^{2+}$ is released from ER through ryanodine receptor channels (RyR2) in a process controlled by several RyR2-associated proteins, including FKBP12.6, triadin, junctin, and calsequestrin. Mice lacking triadin exhibit a large reduction in the amount of junctional ER, resulting in impaired excitation-contraction coupling, whereas calsequestrin deficiency does not have a major effect on excitation-contraction coupling but does promote arrhythmias (Knollmann, 2009). $Ca^{2+}$ release through $IP_3R2$ channels is induced by activation of endothelin-1 receptors in atrial myocytes; in this way, endothelin-1 enhances action potential-induced $Ca^{2+}$ transients and improves the efficiency of excitation-contraction coupling (Li et al., 2005). Cardiomyocyte-specific knockout of the *SERCA2* gene results in only moderate heart dysfunction despite a large reduction in the ER $Ca^{2+}$ content in the myocytes (Andersson et al., 2009). It is noteworthy that the cardiac myocytes lacking SERCA2 adapted to the ER $Ca^{2+}$ deficit by increasing $Ca^{2+}$ influx through plasma membrane L-type channels and the $Na^+/Ca^{2+}$ exchanger and by enhancing the responsiveness of myofilaments to $Ca^{2+}$.

The importance of perturbed ER $Ca^{2+}$ regulation in cardiac function is highlighted by the fact that mutations in RyR2 cause inherited forms of several diseases characterized by cardiac arrhythmias and susceptibility to sudden death. One such inherited arrhythmogenic syndrome called catecholaminergic polymorphic ventricular tachycardia (CPVT) is characterized by hypersensitivity of the heart rhythm to exercise or emotional stress (Liu and Priori, 2008; Betzenhauser and Marks, 2010; Thomas et al., 2010). CPVT is believed to affect approximately 1 in 10,000 individuals. More than 20 mutations in RyR2 have been linked to CPVT, and the mechanism(s) by which these mutations result in disease have been elucidated; in general, the mutations render the RyR2 channel hypersensitive to phosphorylation by PKA, thereby increasing $Ca^{2+}$ release and elevating cytosolic $Ca^{2+}$ levels (Betzenhauser and Marks, 2010). A similar pathogenic mechanism has been proposed for sudden infant death syndrome (Tester et al., 2007).

At least three different molecular events have been proposed to underlie perturbed RyR2 in CPVT. First, studies of CPVT-causing RyR2 mutations were shown to reduce the binding affinity of FKBP12.6 to RyR2, and this effect of the mutations was exacerbated after RyR2 phosphorylation by PKA (Wehrens et al., 2003). However, other studies have revealed that RyR2 mutations do not affect the interaction between FKBP12.6 and RyR2 and that PKA does not dissociate FKBP12.6 from RyR2 mutant channels (George et al., 2003; Jiang et al., 2005; Liu et al., 2006). A second proposed pathogenic mechanism of RyR2 mutations is similar to that proposed for the effects of RyR1 mutations in skeletal muscle cells that result in malignant hyperthermia (see section V). In the closed state, individual RyR2 subunits associate with each other in a so-called zipper domain region, and RyR2 mutations that cause CPVT cause unzipping of these regions, resulting in RyR2 hyperactivation (Lehnart et al., 2005). A third mechanism by which RyR2 mutations may perturb ER $Ca^{2+}$ regulation in CPVT is by increasing the sensitivity of RyR2 to lumenal $Ca^{2+}$ (Jiang et al., 2005). An increased pool of ER $Ca^{2+}$ similar to that caused by presenilin-1 mutations (see section VII) might also contribute to the perturbed $Ca^{2+}$ regulation in cardiac cells caused by RyR2 mutations.

The $Ca^{2+}$-binding protein calsequestrin 2 is present in very high amounts in the ER of cardiac cells, where it is believed to function as a $Ca^{2+}$ buffer (Beard et al., 2004). A few CPVT families have been identified in which the cause of the disease is a recessively inherited mutation in calsequestrin 2 (Lahat et al., 2001). The mutations in calsequestrin 2 may result in a loss of the $Ca^{2+}$-buffering function of the protein (Viatchenko-Karpinski et al., 2004), and/or the mutations may disrupt an interaction between calsequestrin 2 and the RyR2 channel (Terentyev et al., 2006).

Heart failure is a major cause of morbidity and mortality, affecting approximately 5 million Americans, the vast majority of whom are elderly (Rich, 2006). The pathogenesis of heart failure is complex, involving multiple structural and functional alterations, including increased production of oxygen free radicals, impaired excitation-contraction coupling, and deficient force- and relaxation-frequency responses (Janczewski and Lakatta, 2010). Considerable evidence suggests that, among the factors underlying impaired function of cardiomyocytes in heart failure, perturbed ER $Ca^{2+}$ handling may play a particularly important role early in the disease process. Data suggest that heart failure involves hyperphosphorylation of RyR2 by PKA, resulting in excessive $Ca^{2+}$ release and depletion of ER $Ca^{2+}$ stores in cardiac myocytes, associated with an increased diastolic ER $Ca^{2+}$ leak (Yano et al., 2000). Studies of animal models of heart failure have documented an increase in the frequency and duration of ER $Ca^{2+}$ sparks, suggesting more and extended opening of RyR2 channels (Maier et al., 2003). The perturbed ER $Ca^{2+}$ release may impair excitation-contraction coupling.

In addition to perturbed regulation of ER $Ca^{2+}$ release, considerable evidence suggests that a deficiency in SERCA activity occurs in cardiac myocytes in heart failure. The expression of the gene encoding SERCA2, and the overall enzyme activity of SERCA2, are decreased in failing heart cells (Kawase and Hajjar, 2008). In addition, there is evidence that levels of phospholamban, which inhibits SERCA2 activity, is increased in heart failure. Reduced SERCA2 activity would be expected to result in a decreased ER $Ca^{2+}$ pool and delayed restoration of the cytosolic $Ca^{2+}$ concentration after stimulation. In this way, a SERCA2 deficiency would promote diastolic dysfunction and tachycardia. Studies of mice with a genetic deletion of one SERCA2 allele have demonstrated the importance of ER $Ca^{2+}$ uptake in protecting cardiac myocytes against ischemic injury in a model of myocardial infarction (Talukder et al., 2008).

There has been considerable interest in the development of therapeutic interventions aimed at increasing the levels and/or activity of SERCA2 as a treatment for heart failure (Lipskaia et al., 2010). Viral vector-mediated expression of SERCA2a in failing human cardiomyocytes improved their contractility, which was associated with restoration of the $Ca^{2+}$ transient as a result of increased ER $Ca^{2+}$ uptake during diastole and greater $Ca^{2+}$ efflux during systole (del Monte et al., 1999). Overexpression of SERCA2a in a model of cardiac arrhythmia suppressed arrhythmias and also reduced damage to cardiac myocytes (del Monte et al., 2004). Early phase clinical trials of adeno-associated virus-mediated delivery of SERCA2a in patients with heart failure are currently in progress. Small molecules that either enhance SERCA activity or inhibit phospholamban are being developed. For example, istaroxime [(*E*,*Z*)-3-((2-aminoethoxy)imino) androstane-6,17-dione hydrochloride] enhances ER $Ca^{2+}$ uptake in failing cardiomyocytes and improves heart function in a pig model (Micheletti et al., 2007).

### *B. Skeletal Muscle*

Motor neurons release the neurotransmitter acetylcholine, which activates nicotinic acetylcholine receptors in the plasma membrane of skeletal muscle cells, resulting in membrane depolarization and $Ca^{2+}$ influx through voltage-gated L-type channels. The $Ca^{2+}$ influx then activates ryanodine receptors (RyR1) in ER membranes closely apposed to the plasma membrane. $Ca^{2+}$ released from the ER then binds troponin, resulting a conformational change that is transmitted from troponin to tropomyosin, thereby unmasking myosin-binding sites on actin filaments (Rome, 2006). Relaxation occurs when intracellular $Ca^{2+}$ levels recover toward basal levels; recovery of $Ca^{2+}$ levels is mediated in part by ATP-dependent $Ca^{2+}$ uptake into the ER via SERCAs. Skeletal muscle cells predominantly express SERCA1a, although slow-twitch cells also express SERCA2a.

The critical importance of ER $Ca^{2+}$ handling in skeletal muscle cells has been established by the identification of mutations in RyR1 as the cause of inherited disorders that manifest abnormalities in skeletal muscle cells. Malignant hyperthermia is an autosomal-dominant disease characterized by an unusual and dramatic metabolic response to volatile anesthetics, with symptoms that include a rapid rise in body temperature, skeletal muscle contracture, and damage and lysis of muscle cells (Denborough, 1998). More than 80 different missense mutations in the gene encoding RyR1 have been linked to familial malignant hypothermia, accounting for approximately 50% of all cases of the disorder (Treves et al., 2005). The mutations are inherited in an autosomal dominant manner, consistent with a gain-of-function pathogenic action of the mutations. Each mutation consistently increases the sensitivity of RyR1 to opening in response to caffeine and volatile anesthetics such as halothane and also alters excitation-contraction coupling (Tong et al., 1997).

A second autosomal dominantly inherited muscle disorder caused by mutations in RyR1 is called central core disease (CCD); families with recessively inherited CCD caused by RyR1 mutations have also been reported (Jungbluth et al., 2002). CCD is a myopathy present early in life that typically does not progress; patients exhibit hypotonia, proximal muscle weakness, and a developmental delay in motor system maturation. The skeletal muscle cells of patients with CCD exhibit regions devoid of mitochondria called “cores.” RyR1 mutations that cause CCD have been shown to increase RyR1 receptor channel activity (Ghassemi et al., 2009). A third muscle disorder caused by RyR1 mutations is multiminicore disease (MmD), which is inherited in a recessive manner and manifests at birth with hypotonia and distal joint laxity; later in life progressive scoliosis and respiratory insufficiency may develop (Guis et al., 2004). MmD muscle cells exhibit small cores that do not run the length of the muscle fiber. Although the mechanism by which MmD mutations affect the function of RyR1 remains to be established, it seems likely that perturbations in ER $Ca^{2+}$ handling and excitation-contraction coupling are involved.

### *C. Exocrine and Endocrine Systems*

Various hormones are released into the blood from exocrine and endocrine cells in a $Ca^{2+}$-dependent manner. Examples of such hormones include the following: insulin from pancreatic $\beta$ cells; glucocorticoids and epinephrine from adrenal cortical and medullary cells, respectively; vasopressin and oxytocin from axon terminals in the posterior pituitary gland; adrenocorticotropin and gonadotropins from the anterior pituitary; and incretins from intestinal epithelial cells. Because this review focuses on the role of ER $Ca^{2+}$ handling in the physiology and pathophysiology of excitable cells, we will present in this section only examples from exocrine and endocrine cells in which membrane depolarization can elicit an action potential.

Pancreatic $\beta$ cells produce insulin and release it into the blood in response to an elevation of the circulating glucose concentration. Glucose induces electrical activity, first by causing a gradual membrane depolarization to a threshold potential at which action potentials are generated and VGCCs open (Best et al., 2010). Glucose causes membrane depolarization by reducing $K^+$ efflux through K-ATP channels and by opening volume-regulated anion channels. Studies of $Ca^{2+}$ oscillations in mouse $\beta$ cells during glucose stimulation exhibits a descending phase with two components: first, there was a rapid decrease of the cytosolic $Ca^{2+}$ concentration that coincided with closing of VGCCs; second, there was a slower phase that was independent of $Ca^{2+}$ influx (Gilon et al., 1999). When the SERCA was blocked with thapsigargin, the amplitude of the rising phase of cytosolic $Ca^{2+}$ was elevated, and the slow recovery phase was impaired. It is noteworthy that thapsigargin caused depolarization of the plasma membrane, suggesting that $Ca^{2+}$ filling of the ER modulates membrane potential thereby playing a pivotal role in the propagation and maintenance of $Ca^{2+}$ oscillations. It has been suggested that a relatively simple biophysical re-equilibration of $Ca^{2+}$ fluxes can explain such complex patterns of intracellular $Ca^{2+}$ release (Burdakov and Verkhratsky, 2006). Pancreatic $\alpha$-cells are also excitable and release glucagon in response to depolarization and epinephrine. It is noteworthy that in $\alpha$-cells, the initial $Ca^{2+}$ response is due to $Ca^{2+}$ release from the ER, which, in turn, triggers $Ca^{2+}$ -induced $Ca^{2+}$ influx resulting in depolarization and $Ca^{2+}$ influx through VGCCs (Liu et al., 2004). Whereas glucose depolarizes $\beta$ cells, it hyperpolarizes $\alpha$ cells and stimulates $Ca^{2+}$ retention in the ER.

Perturbed ER $Ca^{2+}$ handling may play a role in the pathogenesis of type I diabetes, in which $\beta$ cells become unresponsive to glucose and eventually die. Normally functioning $\beta$ cells exhibit oscillations of intracellular $Ca^{2+}$ levels that are controlled, in part, by ER $Ca^{2+}$ uptake and release (Jahanshahi et al., 2009). Nonoscillatory islet cells exhibit elevated basal cytosolic $Ca^{2+}$ levels and a reduced $Ca^{2+}$ response to glucose. The reason for the defect in $Ca^{2+}$ pulsatility seems to be a reduced pool of releasable ER $Ca^{2+}$ and not an alteration in plasma membrane ion channels. The authors concluded that “Our data suggest the loss of oscillatory capacity may be an early indicator of diminished islet glucose sensitivity and ER dysfunction, suggesting targets to improve islet assessment” (Jahanshahi et al., 2009). In addition to diabetes, the damage of pancreatic cells that occurs in pancreatitis may result, in part, from toxic actions of biliary acids on acinar cells. Two-photon imaging studies from the ER and acidic compartments whithin acinar cells have demonstrated that the biliary acid taurolithocholic acid 3-sulfate causes $Ca^{2+}$ release from both $IP_3$-sensitive and ryanodine-sensitive stores (Gerasimenko et al., 2006).

Anterior pituitary cells produce one or more peptide hormones in response to signals from the brain. For example, pituitary cells that produce adrenocorticotropin are stimulated by corticotropin-releasing hormone, which is produced in hypothalamic neurons in response to stress. The release of adrenocorticotropin is mediated by $Ca^{2+}$ released from $IP_3$-sensitive ER stores and subsequent opening of store-operated $Ca^{2+}$ channels in the plasma membrane (Yamashita et al., 2009). Adrenocorticotropin secretion is blocked by thapsigargin pretreatment, by inhibitors of store-operated $Ca^{2+}$ channels [1-(2-(3-(4-methoxyphenyl)-propoxy)-4-methoxyphenylethyl)-1*H*-imidazole (SKF96365) and *N*-propylargylnitrendipine (MRS1845)], and by L-type $Ca^{2+}$ channel blockers (Won and Orth, 1995; Yamashita et al., 2009).

In addition to glucocorticoids, the adrenal gland produces epinephrine, a second major hormone involved in the response of the body and brain to stress. Epinephrine is produced by neurosecretory cells called chromaffin cells located in the medulla (middle) of the adrenal gland. A study that employed laser microscopy and amperometry showed that chromaffin cells contain both $IP_3$- and ryanodine-sensitive ER $Ca^{2+}$ pools; agonist coupled to $IP_3$ production released approximately twice the amount of $Ca^{2+}$ released in response to caffeine (Inoue et al., 2003). Muscarine-induced $Ca^{2+}$ responses lasted for 10 to 20 s, whereas caffeine-induced $Ca^{2+}$ responses lasted only 3 to 6 s.

### *D. Nervous System*

Neurons represent a unique cell type with a complex morphology that includes a soma, arborized dendrites, dendritic spines, axons, and axon terminals (Fig. 3). The ER extends throughout these distinct compartments and supports functionally diverse roles within each, thereby earning the status of a "neuron-within-a-neuron" (Berridge, 1998, 2002). There is believed to be a single continuous ER store, providing the extensive continuum necessary for synchronization across the distinct spatial and functional compartments of the neuron (Terasaki et al., 1994; Park et al., 2008). For example, in the dendrites, ER $Ca^{2+}$ release is involved in modulating postsynaptic responses and synaptic plasticity

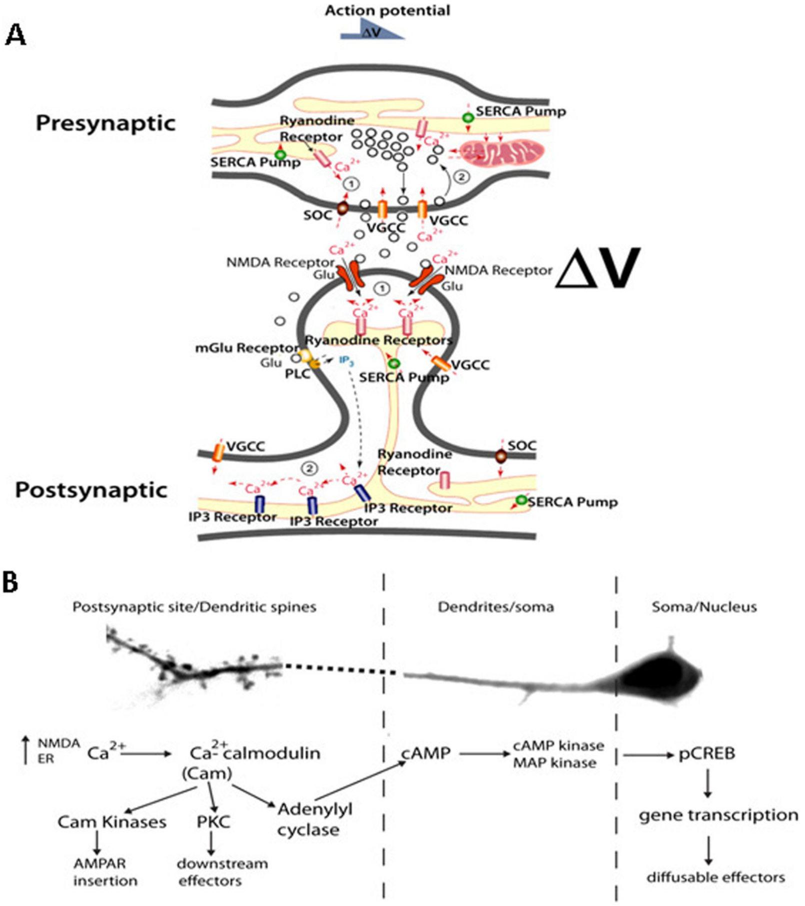

FIG. 3. The role of ER $Ca^{2+}$ in synaptic plasticity. A, the ER can extend into both the pre- and postsynaptic compartments of a synapse. In presynaptic terminals, ER $Ca^{2+}$ release can trigger spontaneous neurotransmitter release and can also integrate with voltage-gated $Ca^{2+}$ entry elicited from action potential invasion to facilitate vesicle release and the repopulation of the ready-releasable pool of vesicles. NMDAR-mediated $Ca^{2+}$ signals are amplified postsynaptically by RyR in dendritic spines and contribute to homosynaptic plasticity. At extrasynaptic sites, glutamate spillover triggers metabotropic glutamate (mGlu) receptor-mediated generation of $IP_3$ and activates a $Ca^{2+}$ response outside of the synaptic contact point. Subsequent activation of $IP_3Rs$ supports regenerative $Ca^{2+}$ waves, which may be involved in heterosynaptic plasticity and gene expression. [Modified from Bardo S, Cavazzini MG, and Emptage N (2006) The role of endoplasmic reticulum $Ca^{2+}$ store in the plasticity of central neurons. *Trends Pharmacol Sci* **27**:78–84.). B, the $Ca^{2+}$ generated by both plasma membrane $Ca^{2+}$-permeable channels (e.g., NMDAR) and ER $Ca^{2+}$ channels can subsequently trigger multiple $Ca^{2+}$-dependent cascades that encode long-term plasticity. In the case of LTP, $Ca^{2+}$ in dendritic spines locally activates effectors, including calmodulin, which in turn activates several kinase pathways such as adenylyl cyclase, CamKII, and PKC. These then trigger longer term cascades, such as the cAMP/phosphorylated cAMP response element-binding protein (pCREB) pathway, which results in protein translation and long-term structural and functional alterations to the neuron that support learning and memory encoding. AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor.

(Emptage et al., 1999; Fitzjohn and Collingridge, 2002; Holbro et al., 2009); in axon terminals, it is involved in vesicle fusion and neurotransmitter release (Emptage et al., 2001; Bouchard et al., 2003); in the soma, it is coupled to the activation of $Ca^{2+}$-sensitive signaling pathways such as kinase and phosphatase activities (Berridge, 1998); and in the perinuclear space, it can trigger gene transcription (Li et al., 1998). Local variations in ER morphology also correlate with dendritic spine density and maturation, linking ER morphology to changes in synaptic organization and function (Harris, 1999; Holbro et al., 2009).

One of the most complex aspects of neuronal communication is the feature of electrochemical synaptic transmission, which is a $Ca^{2+}$-dependent phenomenon that recruits ER stores under a variety of conditions. Although much of the $Ca^{2+}$ entry into the neuron is predominantly mediated by plasma membrane ligand-gated channels (such as NMDA receptors) or VGCCs; $IP_3R$- and/or RyR-mediated $Ca^{2+}$ release can be subsequently recruited via CICR (Finch et al., 1991; Friel and Tsien, 1992). The dynamic interplay between intra- and extracellular $Ca^{2+}$ sources becomes particularly relevant when considering pre- and postsynaptic mechanisms underlying neurotransmission and synaptic plasticity (Berridge, 1998; Verkhratsky, 2002; Park et al., 2008).

RyR are found throughout the neuron, including presynaptic terminals, where CICR can trigger spontaneous neurotransmitter release via coupling of $Ca^{2+}$-binding sensors to neurotransmitter vesicles (Emptage et al., 2001; Bouchard et al., 2003). In addition, ER $Ca^{2+}$ can facilitate subsequent vesicle release by mobilizing neurotransmitter vesicles from the reserve pool to the readily releasable pool. This occurs when presynaptic $Ca^{2+}$ levels are elevated in response to VGCC activity (such as an incoming action potential), and then the readily releasable vesicle pool is released into the synaptic cleft, and vesicles are replenished with neurotransmitter from a reserve pool in a $Ca^{2+}$-dependent manner. RyR-mediated CICR can facilitate this process and thereby accelerate the rate of successful repetitive neurotransmission (Kuromi and Kidokoro, 2002; Zucker and Regehr, 2002). This $Ca^{2+}$-dependent phenomenon influences short-term presynaptic plasticity, such as paired-pulse facilitation, which reflects residual $Ca^{2+}$ remaining in the presynaptic terminal and serves to increase the probability of neurotransmitter release (Zucker and Regehr, 2002; Bouchard et al., 2003). In this phenomenon, ER stores can be a source for the residual $Ca^{2+}$ contributing to paired-pulse facilitation (Emptage et al., 1999). Another form of presynaptic plasticity, post-tetanic potentiation, reflects enhanced neurotransmitter release that briefly (seconds to minutes) leads to synaptic strengthening. RyR-mediated $Ca^{2+}$ release contributes to the residual $Ca^{2+}$ levels via CICR and facilitates post-tetanic potentiation (Zucker and Regehr, 2002; Bardo et al., 2006).

ER $Ca^{2+}$ is involved in several postsynaptic long- and short-term physiological processes. $Ca^{2+}$ partly regulates activity-dependent membrane excitability-sensitive $K^+$ channels, such as the SK channel, which contributes to the medium afterhyperpolarization. This current underlies spike-frequency adaptation, a phenomenon wherein accumulating $Ca^{2+}$ entering though spiking activity reaches sufficient levels to activate hyperpolarizing $K^+$ currents and transiently suppress membrane excitability. Although these channels are largely triggered by VGCC, $IP_3$- and RyR-mediated $Ca^{2+}$ release can also activate these channels and modify spiking patterns, thereby influencing local circuit activity (Stutzmann et al., 2003; Hagenston et al., 2008; Chakroborty et al., 2009). In hippocampal and cortical pyramidal neurons, the ER in the soma and dendritic shafts express both $IP_3R$ and RyR, whereas ER networks in distal processes and dendritic spine heads express a greater proportion of RyR (Sharp et al., 1993; Fitzjohn and Collingridge, 2002; Hertle and Yeckel, 2007). This suggests that $Ca^{2+}$ signaling involving these individual receptors may support different roles in synaptic activity. The somatic $IP_3Rs$ may be involved in gene transcription and protein synthesis, whereas extrasynaptic $IP_3R$ activation may be recruited with synaptic spillover events or require much higher threshold inputs (Nakamura et al., 1999; Mellström and Naranjo, 2001). In contrast, RyRs in dendritic spine heads may be better positioned to modulate incoming synaptic activity directly. For example, in dendritic spines of hippocampal CA1 neurons, the NMDAR-mediated $Ca^{2+}$ signal is largely amplified by RyR-mediated CICR (Alford et al., 1993; Emptage et al., 1999).

The CICR-mediated enhancement of $Ca^{2+}$ signals initiated by plasma membrane $Ca^{2+}$ channels plays an important role in synaptic transmission and synaptic plasticity—the cellular mechanism by which learning and memory are thought to be encoded (Ross et al., 2005; Watanabe et al., 2006). Most commonly, synaptic plasticity is initiated within dendritic spines, which express several $Ca^{2+}$ permeable channels, such as NMDAR, VGCCs, RyR, and $IP_3R$ (Yuste et al., 2000; Yasuda et al., 2003). Although NMDAR-mediated $Ca^{2+}$ entry is often necessary for LTP induction, this $Ca^{2+}$ source alone is not sufficient to sustain long-term forms of plasticity (Raymond and Redman, 2006). ER $Ca^{2+}$ stores are essential to this process by amplifying and extending the duration of the initial NMDAR-mediated signal and ensuring the proper spatial and temporal $Ca^{2+}$ patterns necessary to activate the specific downstream cascades necessary to encode LTP or LTD. Therefore, manipulating the ER $Ca^{2+}$ channels greatly affects the expression of plasticity. For example, the polarity and input specificity of long-term plasticity has been shown to be regulated by ER $Ca^{2+}$ stores such that blocking $IP_3R$ leads to a conversion of LTD to LTP and elimination of heterosynaptic LTD, whereas blocking RyR eliminates homosynaptic LTD and LTP induction (Obenaus et al., 1989; Harvey and Collingridge, 1992; Nishiyama et al., 2000; Fitzjohn and Collingridge, 2002; Chakroborty et al., 2009).

Additional evidence for the fundamental role of RyR in synaptic plasticity emerges from studies using RyR knockout mice. For example, RyR3 knockout mice show enhanced LTP, which is independent of NMDAR-mediated mechanisms, but impaired LTD (Futatsugi et al., 1999); the RyR3 is expressed in dendritic processes of hippocampal neurons (Hertle and Yeckel, 2007), suggesting that RyR3 isoform may function to suppress LTP and facilitate LTD. This may in turn serve to maintain the balance of excitation and inhibition that determines the overall stability of the synapse. There also seems to be functional overlap between RyR- and $IP_3R$-mediated $Ca^{2+}$ signaling and plasticity. Type 1 $IP_3R$ knockout mice also demonstrate enhanced LTP, whereas LTD is not affected (Fujii et al., 2000), suggesting that $IP_3R$-sensitive ER $Ca^{2+}$ stores in general have an inhibitory role in LTP induction. Furthermore, $IP_3R$-mediated $Ca^{2+}$ stores outside dendritic spines may also suppress LTP in neighboring synapses, thus maintaining the input specificity that is characteristic of LTP. These and related studies demonstrate that ER $Ca^{2+}$ is required for neuronal synaptic plasticity and, by association, supports memory and cognitive functions (Fig. 3).

## V. Perturbed Endoplasmic Reticulum $Ca^{2+}$ Handling and Disease

### A. *Ischemic Stroke*

Ischemic stroke results when a clot forms in a cerebral blood vessel that, depending upon the vessel affected and for how long, results in varying amounts of morbidity or mortality. Stroke is a leading cause of death worldwide; risk factors include hypertension, obesity, diabetes, and smoking. More than any other cell type, neurons are exquisitely vulnerable to ischemia because of their high energy demand, their reliance on glucose as an energy source, and their excitability and sensitivity to the excitatory neurotransmitter glutamate (for review, see Mattson, 2003). Cellular $Ca^{2+}$ overload is strongly implicated in the degeneration and death of neurons that occurs in ischemic stroke; studies of experimental models indicate that $Ca^{2+}$ influx through NMDA receptors and VGCCs can be pivotal in such ischemic neuronal death (Verkhratsky and Toescu, 2003; MacDonald et al., 2006; Mattson, 2007). The pharmacology of glutamate and VGCC in relation to stroke and excitotoxic neuronal death has been reviewed in detail previously (Catterall et al., 2005; Traynelis et al., 2010). In this section, we focus instead on the role of perturbed ER $Ca^{2+}$ handling in stroke, and the potential of agents that target ER $Ca^{2+}$ regulation in stroke therapy.

Evidence for the involvement of ER $Ca^{2+}$ handling systems in ischemic stroke comes from studies demonstrating changes in ER $Ca^{2+}$ release or uptake in experimental models relevant to stroke. Release of $Ca^{2+}$ from caffeine/ryanodine-sensitive stores occurs before the death of CA1 hippocampal neurons in a model of global cerebral ischemia—reperfusion injury (Xing et al., 2004). Dantrolene, which inhibits $Ca^{2+}$ release from RyR, reduced brain damage in animal models of neonatal and adult hypoxia/ischemia (Wei and Perry, 1996; Gwak et al., 2008). Another study employed a model in which cultured hippocampal neurons were exposed to the glycolysis inhibitor iodoacetate, which causes a slowly progressing cell death that is exacerbated by caffeine, and 1 $\mu$M caffeine, which activates RyR (Hernández-Fonseca and Massieu, 2005). Dantrolene and a higher concentration of ryanodine (25 $\mu$M), which antagonizes RyR, attenuated neuronal death in iodoacetate-treated cultures.

ER $Ca^{2+}$ overload impairs protein synthesis, and unfolded proteins accumulate in the ER lumen (Paschen, 2004). This accumulation of unfolded proteins in the ER can trigger two molecular stress responses: 1) UPR, which is required for inducing the new synthesis of chaperones to refold the unfolded proteins, and 2) ER-associated degradation, which targets damaged proteins for degradation in the proteasome. If sufficient synthesis of the ER chaperone GRP78 occurs, the unfolded proteins may be refolded, and the triggering of apoptotic cell death avoided (Yu et al., 1999).

In addition to impaired SERCA activity and enhanced release of $Ca^{2+}$ through $IP_3$ and RyR, data suggest a role for presenilin-1 in ischemic neuronal injury. Thus, neurons in presenilin-1 mutant knockin mice exhibit increased vulnerability to focal ischemic stroke and an instability of ER $Ca^{2+}$ homeostasis under hypoxic and energetic stress (Mattson et al., 2000). Capacitative $Ca^{2+}$ entry may play an important role in ischemic neuronal death, because STIM1 is essential for capacitive $Ca^{2+}$ entry and ischemia-induced $Ca^{2+}$ overload in neurons (Berna-Erro et al., 2009). Neurons from STIM2-deficient mice showed significantly increased survival under hypoxic conditions compared with neurons from wild-type mice. It has been proposed that ischemia enhances *S*-glutathionylation of RyR, which allows RyR to sustain CICR, resulting in increased vulnerability of neurons to $Ca^{2+}$ overload and cell death (Bull et al., 2008).

Although $Ca^{2+}$ release from the ER may contribute to ischemic neuronal death, it may also play an important role in ischemic preconditioning hormesis (Bickler et al., 2009), a process in which exposure of neurons to a mild brief ischemia results in resistance to a more severe ischemic stroke (Calabrese et al., 2007). Transient anoxia has been shown to activate the transcription factor NF-$\kappa$B, resulting in increased expression of $Na^+/Ca^{2+}$ exchanger 1, which, in turn, enhances $Ca^{2+}$ refilling

(Sirabella et al., 2009). Other studies have found that NF-κB can enhance whole-cell $Ca^{2+}$ currents while down-regulating NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid currents in hippocampal neurons (Furukawa and Mattson, 1998). It is noteworthy that ER $Ca^{2+}$ release can be a stimulus for NF-κB activation. Inhibition of $Ca^{2+}$ release via $IP_3R$ channels decreases basal NF-κB activity in cultured rat cortical neurons (Glazner et al., 2001). Moreover, activation of NF-κB in response to TNF and glutamate is abolished in neurons treated with an $IP_3R$ inhibitor. Additional findings suggest that a factor, probably a protein, released from the ER when $IP_3R$ are activated is responsible for activation of NF-κB (Glazner et al., 2001).

### *B. Lipid Storage Disorders: Gaucher, Sandhoff, and Niemann-Pick C Diseases*

Deficiencies in enzymes involved in cellular lipid metabolism can result in diseases that involve neuronal dysfunction and degeneration. Gaucher disease is caused by deficiency of lysosomal glucocerebrosidase activity and accumulation of glucosylceramide, a glucocerebrosidase substrate. Mutations in glucocerebrosidase may destabilize its structure, resulting in misfolding and degradation of the enzyme (for review, see Vitner et al., 2010). The accumulation of glucosylceramide results in $Ca^{2+}$ release through ER RyR, and blockade of RyR can restore normal folding of mutant glucocerebrosidase in fibroblasts from patients with Gaucher disease (Wang et al., 2011). Likewise, Ong et al. (2010) found that increasing ER $Ca^{2+}$ levels by reducing ER $Ca^{2+}$ efflux through RyR (with the use of antagonists or RNA interference) or by enhancing ER $Ca^{2+}$ influx through SERCA2b overexpression increased glucocerebrosidase activity in fibroblasts from patients with Gaucher disease. There may be an increase in the size of the ER $Ca^{2+}$ pool in Gaucher disease, because treatment of cultured hippocampal neurons with an inhibitor or glucosylceramidase results in increased density of ER and greater $Ca^{2+}$ responses to glutamate and caffeine (Korkotian et al., 1999).

Sandhoff disease is caused by mutations in the β-chain of hexosaminidase, resulting in deficiency of hexosaminidases A and B, resulting in the intracellular accumulation of GM2 ganglioside (Kolter and Sandhoff, 2005). Patients with Sandhoff disease exhibit progressive neurological deficits that include developmental delay, gait disturbances, and speech impairment (Maegawa et al., 2006). Hexosaminidase B-deficient mice exhibit GM2 accumulation in their brain cells, and microsomes prepared from Hexb(−/−) mouse brain exhibit a reduced rate of $Ca^{2+}$-uptake via the SERCA that can be prevented by feeding the mice *N*-butyldeoxynojirimycin, an inhibitor of glycolipid synthesis that reduces GM2 storage (Pelled et al., 2003). Neurons cultured from embryonic Hexb(−/−) mice exhibit increased sensitivity to death induced by thapsigargin. The reduced SERCA activity and increased sensitivity to ER $Ca^{2+}$ store depletion may contribute to the neuronal dysfunction and degeneration that occurs in Sandhoff disease. Overexpression of hexosaminidase B accomplished with the use of a bicistronic lentiviral vector can normalize the ER $Ca^{2+}$ uptake defect and decrease GM2 in hippocampal neurons from embryonic Sandhoff mice (Arfi et al., 2006).

Niemann-Pick type C disease (NPC) is an inherited lipid storage disorder caused by deficiencies of lysosomal proteins (NPC1 and NPC2) involved in intracellular cholesterol-trafficking. Patients with NPC exhibit progressive neurological impairment and die at an early age; cerebellar Purkinje cells are particularly vulnerable (Tang et al., 2010). NPC1 mutations result in impaired $Ca^{2+}$-mediated fusion of endosomes with lysosomes, resulting in the accumulation of cholesterol and other lipids in late endosomes and lysosomes. Preclinical studies suggest that cyclodextrin, an agent known to reduce cholesterol accumulation in cells, can stimulate lysosomal exocytosis in a $Ca^{2+}$-mediated manner (Chen et al., 2010). It was reported that NPC1 mutant fibroblasts have a much reduced level of acidic compartment calcium stores compared with wild-type control cells (Lloyd-Evans et al., 2008). When luminal endocytic calcium was chelated in normal cells with high-affinity rhod-dextran, the cells exhibited an NPC-like disease phenotype. In another model, the same authors found that excessive sphingosine storage in the acidic compartment resulted in calcium depletion and increased cholesterol accumulation in the same compartment (Lloyd-Evans et al., 2008).

### *C. Peripheral Neuropathies and Amyotrophic Lateral Sclerosis*

A common neurological complication of long-standing diabetes is peripheral neuropathy (PN), a condition that involves sensory neurons and typically results in severe pain (Tavakoli and Malik, 2008). Evidence for the involvement of perturbed $Ca^{2+}$ regulation in peripheral nerve cells in PN has been reviewed (Fernyhough and Calcutt, 2010). ER $Ca^{2+}$ signaling is altered in sensory neurons in animal models of PN. Diabetes results in a reduction in the ER $Ca^{2+}$ content in sensory neurons, which, in turn, reduces the amount of $Ca^{2+}$ released upon stimulation by ATP (via activation of purinergic receptors coupled to $IP_3$ production) or caffeine. The impaired $Ca^{2+}$ release was more prominent in dorsal root ganglion neurons of the lumbar region compared with those in the cervical and thoracic regions (Huang et al., 2002). In the rat streptozotocin-induced diabetes model, fluorescence video imaging was used to measure free cytosolic $Ca^{2+}$ levels in lumbar nociceptive neurons of control and diabetic rats. The basal $Ca^{2+}$ concentration in the neurons rose progressively with the duration of diabetes, and $Ca^{2+}$ mobilization from ER $IP_3$- and ryanodine-sensitive $Ca^{2+}$ stores was reduced in sensory neurons of the diabetic rats (Kruglikov et al., 2004). In a similar diabetes model, the soleus muscle exhibited decreased SERCA2a levels in type I (slow twitch) fibers compared with nondiabetic control rats (Rácz et al., 2009).

ALS is a fatal neurodegenerative disorders in which lower and upper motor neurons degenerate, resulting in progressive paralysis. Some cases of ALS are caused by mutations in Cu/Zn-SOD, and transgenic mice that express mutant human Cu/Zn-SOD provide a model that resembles the human disease (DiBernardo and Cudkowicz, 2006). Studies of Cu/Zn-SOD mutant mice and spinal cords of patients with ALS have provided evidence that motor neurons die as the result of increased oxidative stress, excessive activation of glutamate receptors, and cellular $Ca^{2+}$ overload (Kruman et al., 1999; Guo et al., 2000). Release of $Ca^{2+}$ from the ER is believed to contribute to motor neuron degeneration (Grosskreutz et al., 2010). The mechanism underlying the perturbed $Ca^{2+}$ homeostasis in motor neurons may involve impaired ability of astrocytes to remove glutamate from the extracellular fluid (Rothstein, 2009). In addition, it has been proposed that some cases of ALS involve an autoimmune attack on motor neurons, mediated by antibodies against VGCC (Engelhardt et al., 1995). The reason that some motor neurons in the brainstem do not degenerate in ALS is not known, but those resistant neurons express much higher levels of $Ca^{2+}$-binding proteins such as calbinin (Grosskreutz et al., 2010) that are known to protect neurons against excitotoxicity (Mattson et al., 1991). A dominantly inherited mutation in the vesicle-associated membrane protein-associated protein B (VAPB) is responsible for some cases of ALS. Expression of mutant VAPB in motor neurons results in ER stress and dysregulation of ER and cellular $Ca^{2+}$ homeostasis, and this abnormal $Ca^{2+}$ handling plays a pivotal role in the death of motor neurons caused by the mutant VAPB (Langou et al., 2010). Collectively, the available data suggest a role for excessive elevation of intracellular $Ca^{2+}$ levels in the degeneration of neurons in ALS, although the contribution of specific alterations in ER $Ca^{2+}$ handling systems in this disease is unknown.

### *D. Parkinson Disease*

Parkinson disease (PD), the most common movement disorder, is characterized by degeneration of monoaminergic neurons in the brainstem and basal ganglia, loss of dopaminergic neurons in the substantia nigra playing a major role in the motor symptoms. Although most cases of PD are sporadic, some families harbor mutations that result in inherited early-onset PD. The genetic abnormalities include mutations in genes inherited in either an autosomal dominant (α-synuclein and LRRK2) or recessive (Parkin, DJ-1, and PINK1) (Dawson et al., 2010). Several findings suggest that dopaminergic neurons die as the result of mitochondrial stress, with a possible role for perturbed $Ca^{2+}$ homeostasis downstream of the mitochondrial alterations (Mattson et al., 2008). Studies of cultured cells and transgenic mice expressing mutant α-synuclein, LRRK2, Parkin and DJ-1 implicate the involvement of proteotoxic and oxidative stress in the ER and mitochondria in PD. As a result of the mutations and the aging process, $Ca^{2+}$ handling in the ER and mitochondria may be disturbed (Chan et al., 2009). Cybrid cells containing mitochondria from patients with PD recover from $IP_3$-induced $Ca^{2+}$ release more slowly than control subjects, a behavior similar to that seen in cells exposed to 1-methyl-4-phenylpyridinium ion (Sheehan et al., 1997). It would seem that mitochondrial alterations secondarily affect ER $Ca^{2+}$ handling in this model. One protein that may protect the ER against aging and PD is called Herp (homocysteine-inducible ER stress protein), an integral membrane protein containing a ubiquitin-like domain. Knockdown of Herp increases, and overexpression of Herp decreases, the vulnerability of dopamine-producing cells to the toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Herp prevents ER $Ca^{2+}$ store depletion and mitochondrial $Ca^{2+}$ accumulation by a mechanism requiring proteasomal degradation (Chigurupati et al., 2009).

### *E. Alzheimer Disease*

The etiology of AD is currently unknown, but the classic diagnostic features are well categorized. Histological features include amyloid β-peptide (Aβ) plaques, neurofibrillary tangles composed of hyperphosphorylated τ, and cell death. Behavioral features include emotional and affective changes that may precede the devastating progressive and irreversible memory loss. It is noteworthy that neither of the histopathological markers correlates well with the cognitive changes; instead, decrements in dendritic spine density and synaptic integrity are better associated with memory loss (Goldman et al., 2001; Selkoe, 2002; Scheff et al., 2006). This makes functional sense, because synapses are the sites in which learning and memory are encoded; a loss of synaptic function would therefore impair these cognitive functions. What underlies these synaptic changes is thus a highly relevant, and currently unknown, critical question that must be answered to understand AD pathogenesis.

Dysregulated $Ca^{2+}$ signaling has been given increasing attention as a significant contributing factor in AD, both in early and late stages of the disease (LaFerla, 2002; Stutzmann, 2007; Bezprozvanny and Mattson, 2008). Exquisitely controlled $Ca^{2+}$ levels are fundamental to neuronal functioning and viability, and neurons enlist a host of $Ca^{2+}$ buffers, binding proteins, pumps, and sequestering mechanisms to maintain proper homeostasis. Alterations in $Ca^{2+}$ levels can therefore lead to a variety of neurodegenerative diseases. Specific to AD, it has been shown that sustained up-regulation of

$Ca^{2+}$ levels can both initiate and accelerate the core diagnostic features—from amyloid plaque deposition to synapse loss (Stutzmann, 2007).

AD can be grouped into two categories; the most common is termed sporadic AD, with an unknown etiology and a late age of onset (>65 years). The relatively less common form (1–10% of cases) is termed familial or early-onset AD, and is caused by mutations in the presenilin 1 (*PS1*), presenilin 2 (*PS2*), or amyloid precursor protein (*APP*) genes, and is inherited in an autosomal dominant fashion. Regardless of the form, the disease progression follows the same course, albeit at an accelerated rate in familial cases. Mutations in PS1 are responsible for the majority of FAD. PS is located in the ER membrane and is part of the $\gamma$-secretase complex that cleaves APP into $A\beta_{40}$ or $A\beta_{42}$ peptide fragments, the latter being the most pathogenic form of $A\beta$ that preferentially contributes to plaque formation. PS mutations may cause early-onset AD, potentially through more than one mechanism. One mechanism is that the mutation results in the preferential cleavage of APP into the more amyloidogenic $A\beta_{42}$ form, and a second mechanism is that the mutation results in increased $Ca^{2+}$ release from the ER (Mattson, 2004). It is noteworthy that the altered $Ca^{2+}$ signaling is present early in development, long before the onset of measurable histopathology or cognitive deficits. Previous studies in human fibroblasts from asymptomatic FAD patients and in model cells demonstrated that expression of mutant PS1 or PS2 generated enhanced $IP_3R$-evoked $Ca^{2+}$ responses (Ito et al., 1994). This was later validated in cultured neuronal-like cells expressing mutant PS1 (Guo et al., 1996, 1997; Cheung et al., 2010), in cultured primary neurons from PS1 mutant knockin mice (Guo et al., 1999a; Chan et al., 2000), and in brain slice preparations from young, adult, and aged mutant PS1-expressing mice (Stutzmann et al., 2006; Goussakov et al., 2010).

Perturbed ER $Ca^{2+}$ handling has been shown to mediate several adverse effects of PS1 mutations on neurons. For example, hippocampal neurons from PS1 mutant knockin mice exhibit increased vulnerability to excitotoxicity that is associated with excessive elevations of intracellular $Ca^{2+}$ levels; treatment of the neurons with dantrolene can protect them against the adverse effect of the PS1 mutation (Guo et al., 1999b). PS1 mutations also increase the vulnerability of neurons to mitochondrial impairment, again by a mechanism involving $Ca^{2+}$ release from the ER (Keller et al., 1998). The combination of increased $A\beta42$ production and excessive ER $Ca^{2+}$ release may explain the very early age of disease onset in those who inherit a PS1 mutation.

The mechanism by which mutant presenilin alters ER $Ca^{2+}$ release is still under investigation, and current studies focus on the $IP_3R$, the RyR, and the ER leak channel, as well as interactions among these. The earliest studies identifying a link between mutant PS and ER $Ca^{2+}$ release relied on $IP_3R$ agonists and thereby implicated the $IP_3R$ as the target $Ca^{2+}$ channel (Ito et al., 1994; Leissring et al., 1999). More recent studies have provided a mechanism by which this can occur, such that mutant PS alters the properties of the $IP_3$ channel by increasing the open probability at low cytosolic $[IP_3]$ and shifting the channel gating toward a high open-probability burst mode (Cheung et al., 2008, 2010). This results in a greater $IP_3$-evoked $Ca^{2+}$ response even at low concentrations of circulating $IP_3$. Consistent with this, experiments in mutant PS-expressing mice demonstrate that baseline levels of endogenous $IP_3$ are sufficient to trigger an $IP_3R$-$Ca^{2+}$ response upon increased cytoplasmic $Ca^{2+}$ levels via RyR activation (Goussakov et al., 2010).

In addition to $IP_3R$-mediated changes, RyR-mediated increases in $Ca^{2+}$ release have also been implicated as an underlying factor. Initial studies in cultured neurons from mutant PS mutant mice demonstrated up-regulated RyR expression levels (Chan et al., 2000), and increased RyR-evoked calcium release in cultured cells (Smith et al., 2005; Zhang et al., 2010). Studies in brain slice preparations have also revealed increased RyR-evoked $Ca^{2+}$ responses across specific neuronal compartments, including the soma and perinuclear regions, and particularly high release in dendrites and spine heads (Stutzmann et al., 2006; Goussakov et al., 2010). In asymptomatic young mice, this was associated with an increase in the RyR2 isoform (Chakroborty et al., 2009), whereas increased RyR3 expression has been observed at later disease stages concurrent with $A\beta_{1-42}$ expression (Supnet et al., 2006). Although mutant PS1-expressing mice seem to be cognitively and neurophysiologically normal at this younger, presymptomatic age (Oddo et al., 2003), upon manipulation of the RyR-sensitive stores, it is apparent that these neurons are using a markedly different $Ca^{2+}$ signaling system to support neurotransmission and plasticity (Chakroborty et al., 2009). This suggests that a compensatory homeostatic mechanism is used in presymptomatic brains to maintain normal basal synaptic transmission as well as long- and short-term forms of plasticity. The long-term effects of maintaining this homeostasis are presently unclear but over a period of many years may influence the course of the disease process. Indeed, it was recently reported that as PS1 mutant knockin mice age, they develop a deficit in late LTP in synapses in cornu ammonis field 1 of the hippocampus (Auffret et al., 2010). Moreover, activation of muscarinic receptors, which normally enhances LTP at synapses in cornu ammonis field 1 of the hippocampus, impairs LTP in PS1 mutant knockin mice; the impaired LTP is associated with a reduction in NMDA current that is restored by intracellular $Ca^{2+}$ chelation (Wang et al., 2009). Additional findings in the latter study revealed similar abnormalities in acetylcholine- and NMDA receptor-mediated components of synaptic plasticity in 3xTgAD mice with PS1, APP, and $\tau$ mutations, suggesting that the adverse effects of mutant

PS1 on synaptic plasticity can occur in the absence or presence of pathological amyloid and $\tau$.

Another proposed mechanism by which mutant PS results in altered ER $Ca^{2+}$ signaling involves the ER $Ca^{2+}$ leak channel. The presence of an ER leak channel has primarily been inferred by blocking the SERCA pumps and observing the passive $Ca^{2+}$ leak from the ER, but it has not yet been definitively identified at the molecular or channel level. One hypothesis posits that presenilin functions, in part, as the leak channel and contributes to the maintenance of optimal ER $Ca^{2+}$ levels. AD-linked mutations in presenilin impair its leak properties and thereby result in increased ER $Ca^{2+}$ store levels (Tu et al., 2006; Nelson et al., 2007). It is noteworthy that there is an apparent correlation between particular FAD-linked presenilin mutations and variants of AD clinical phenotypes (Nelson et al., 2010). Concomitant with the impairment in leak channel function, the increase in RyR expression is thought to be a compensatory and neuroprotective response to assume the leak channel role and normalize ER store levels (Zhang et al., 2010). Other neuroprotective roles of the RyR3 isoform have also been proposed at later disease stages, such that increased RyR3 expression is observed upon $A\beta_{42}$ exposure, whereas knockdown of RyR3 increases amyloid pathologic condition (Supnet et al., 2006, 2010). Likewise, long-term exposure to RyR blockers increases amyloid pathologic condition and cytotoxicity (Zhang et al., 2010). On the other hand, RyR blockers have been shown to protect neurons against the endangering effects of presenilin mutations in experimental models of excitotoxicity and $A\beta$ toxicity (Guo et al., 1997, 1999b). The RyR-mediated $Ca^{2+}$ signaling alterations do not occur in isolation, but probably reflect an enhanced CICR response, such that the $Ca^{2+}$ threshold for activating a RyR response is greatly reduced in mutant PS neurons. Thus, $Ca^{2+}$ released via $IP_3R$ can drive a markedly enhanced RyR-$Ca^{2+}$ response, as can $Ca^{2+}$ entry through plasma membrane channels such as NMDA receptors in spines (Goussakov et al., 2010). This has more far-reaching implications for pathological synaptic conditions and for NMDA-targeted therapeutic strategies.

Evidence for $Ca^{2+}$-based signaling defects in sporadic AD exist as well; notably, most of the major hallmarks and known genetic risk factors for AD generate some form of $Ca^{2+}$ dysregulation (Stutzmann, 2007; Bezprozvanny and Mattson, 2008). $A\beta$ peptides, the oligomeric species in particular, have been shown to increase intracellular $Ca^{2+}$ levels through a variety of mechanisms, the major underlying themes involving membrane-associated oxidative stress (lipid peroxidation), membrane disruption, and interactions with endogenous $Ca^{2+}$ channels (Mark et al., 1997a,b; Bruce-Keller et al., 1998; Cutler et al., 2004; Demuro et al., 2010). Several studies have demonstrated that $A\beta$ peptides interact with and alter the properties of membrane lipids, thereby increasing permeability to $Ca^{2+}$ and other anions (Müller et al., 1995; Cribbs et al., 1997). With the high electrochemical gradient for $Ca^{2+}$, compromised plasma membranes will preferentially pass $Ca^{2+}$ into the cytosol. Electrophysiological and structural studies have shown separately that $A\beta$ peptides can incorporate into the plasma membrane and form cation-selective high-conductance pores that are capable of disrupting cellular homeostasis (Arispe et al., 1993; Pollard et al., 1993; Lashuel et al., 2002). The clinical relevance of the $A\beta$ pores to AD pathology is supported by their selective presence in the brains of patients with AD and not in healthy subjects (Inoue, 2008). As a third proposed mechanism, $A\beta$ peptides have been shown to interact with several $Ca^{2+}$-permeable channels and to increase $Ca^{2+}$ flux. These include several voltage-gated $Ca^{2+}$ channels, $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and NMDA glutamate receptors, and serotonergic ($5-HT_3$) and cholinergic (nicotinic $\alpha7$ and $\alpha4\beta2$) receptors (Buckingham et al., 2009; Demuro et al., 2010; Verdurand et al., 2011).

Regarding intracellular $Ca^{2+}$ signaling, $A\beta$ specifically disrupts ER $Ca^{2+}$ channels as well, contributing to broader $Ca^{2+}$ signaling disruptions. $A\beta_{42}$ peptides have been shown to increase RyR3 expression in transgenic mouse models of AD (Supnet et al., 2006, 2010) as well as to increase the RyR open channel probability, resulting in increased $Ca^{2+}$ flux (Shtifman et al., 2010). Likewise, $A\beta$ peptides increase the $IP_3$-evoked $Ca^{2+}$ response in neurons directly (Schapansky et al., 2007), as well as indirectly through the alteration of $G_q$-coupled mGlurR5 receptors (Casley et al., 2009; Renner et al., 2010). On the other hand, by impairing coupling of muscarinic acetylcholine receptors to Gq11 via a membrane lipid peroxidation-mediated mechanism, $A\beta$ can suppress $Ca^{2+}$ responses to acetylcholine (Kelly et al., 1996). In a cyclical fashion, it has also been shown that $Ca^{2+}$ can initiate and accelerate the formation of pathogenic $A\beta$ species (Isaacs et al., 2006), but relevant to this topic is that $Ca^{2+}$ from RyR-sensitive stores in particular can enhance the production and release of $A\beta$ peptides (Querfurth and Selkoe, 1994; Querfurth et al., 1997) (Fig. 4). More recently described is a novel $Ca^{2+}$ channel (CALHM1) localized to the ER and plasma membranes. Although the channel's intended function is unclear, mutations in the *CALHM1* gene have been associated with AD and result in increased $A\beta$ formation (Dreses-Werringloer et al., 2008; Boada et al., 2010; Cui et al., 2010; Cui et al., 2010). However, the role of CALHM1 in AD is still under debate, and several studies, albeit possibly underpowered (Boada et al., 2010), have either not supported or have modified the original findings (Bertram et al., 2008; Beecham et al., 2009; Minster et al., 2009; Lambert et al., 2010).

$\tau$ pathology, in the form of hyperphosphorylated $\tau$ aggregates that give rise to intracellular neurofibrillary tangles, is also a diagnostic component of AD. Upon accumulation within neurons, the tangles impair cellu-

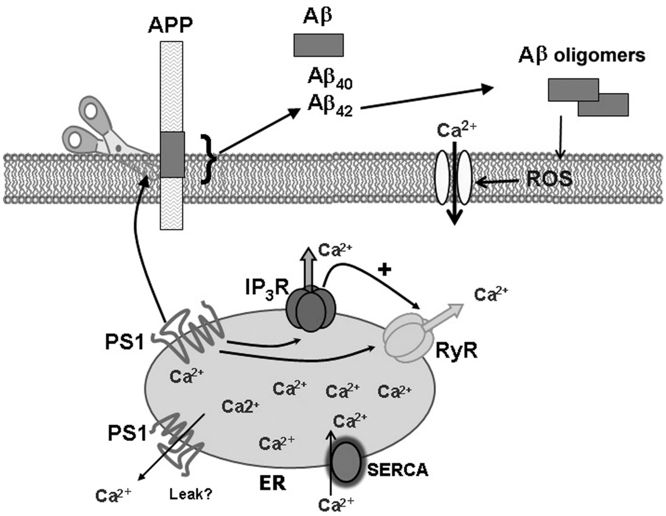

FIG. 4. The role of presenilin in ER Ca$^{2+}$ signaling and dysregulation. The transmembrane-spanning presenilin protein largely functions as an aspartyl protease localized in the ER membrane. It cleaves several type 1 membrane proteins, including $\beta$-APP. As part of the $\gamma$-secretase complex, presenilin cleaves APP (scissors) to generate A$\beta_{40}$ and A$\beta_{42}$. A$\beta_{42}$ readily self-aggregates to form toxic oligomers that may damage neurons by inducing membrane-associated reactive oxygen species (ROS) that, in turn, impair the function of ion-motive ATPases, resulting in membrane depolarization and Ca$^{2+}$ influx through glutamate and voltage-dependent channels. Ca$^{2+}$ oligomers may also form Ca$^{2+}$-conducting pores in the membrane. Presenilin-1 (PS1) mutations that cause Alzheimer disease result in increased levels of A$\beta_{42}$, rather than the more commonly produced and relatively inert A$\beta_{40}$ fragment generated by wild-type PS1. PS1 mutations also result in increased Ca$^{2+}$ release from ER stores through a mechanism that probably involves IP$_3$R and RyR. This increased Ca$^{2+}$ flux also accelerates A$\beta$ formation, which in turn contributes to Ca$^{2+}$ dyshomeostasis. PS1 may also be involved in ER Ca$^{2+}$ homeostasis by serving as a leak channel; and PS1 mutations may impair this Ca$^{2+}$ leak channel function, thereby leading to increased resting Ca$^{2+}$ store levels and increased Ca$^{2+}$ release upon activation of IP$_3$R or RyR.

lar trafficking and synaptic function and ultimately contribute to cell death (Iqbal et al., 2005). Two decades ago, it was reported that excessive elevations of intracellular Ca$^{2+}$ levels, such as occurs during chronic activation of glutamate receptors, can induce $\tau$ hyperphosphorylation and intracellular aggregation in hippocampal neurons similar to the alterations seen in neurofibrillary tangles in AD (Mattson, 1990). Several recent studies elegantly synthesize the pathogenic interrelationship of A$\beta$, $\tau$ phosphorylation, and Ca$^{2+}$, demonstrating how Ca$^{2+}$ elevation, probably resulting from A$\beta$, causes mis-sorting and hyperphosphorylation of $\tau$ into dendrites, and it underlies the breakdown of dendritic spines and synaptic dysfunction (Hoover et al., 2010; Zempel et al., 2010). Many of the kinases that are involved in the pathogenic hyperphosphorylation process are regulated by Ca$^{2+}$, such as glycogen synthase kinase 3$\beta$ and cyclin-dependent kinase 5 (Avila et al., 2004). Increases in cytosolic Ca$^{2+}$ levels, originating from either intra- or extracellular sources, can therefore accelerate the activity of these kinases and facilitate tangle formation. Analogous to the A$\beta$/Ca$^{2+}$ dynamic, phosphorylated or mutant $\tau$ can also increase Ca$^{2+}$ levels within neurons (Gómez-Ramos et al., 2006), maintaining the feed-forward degenerative cycle between Ca$^{2+}$ dysregulation and AD progression.

Although the cause(s) of sporadic late-onset AD are presently undefined, some genetic and environmental risk factors have been identified. *CALHM1* mutations were already mentioned, but expression of two apolipoprotein E $\varepsilon$4 alleles is one of the better studied examples and has been shown to increase the likelihood of developing sporadic AD by 15-fold (Farrer et al., 1997). Apolipoprotein E primarily functions in cholesterol and lipid transport, but the $\varepsilon$E4 variant also impinges on Ca$^{2+}$ signaling pathways and can alter intracellular Ca$^{2+}$ levels through several pathways. These include up-regulation of NMDA-mediated Ca$^{2+}$ influx, recruitment of intracellular stores and voltage-sensitive plasma membrane channels, and rises in resting Ca$^{2+}$ levels (Tolar et al., 1999; Ohkubo et al., 2001; Qiu and Gruol, 2003). A sedentary lifestyle, excessive energy intake, and diabetes may increase the risk of AD (for review, see Kapogiannis and Mattson, 2011). Other studies have shown that exercise and dietary energy restriction can protect neurons against insults that disrupt cellular Ca$^{2+}$ homeostasis, including overactivation of glutamate receptors, mitochondrial impairment, ischemia, and A$\beta$ (Bruce-Keller et al., 1999; Halagappa et al., 2007; Arumugam et al., 2010). The impact of exercise and dietary energy intake on ER Ca$^{2+}$-regulating systems remains to be determined; however, it was reported that dietary energy restriction results in up-regulation of the ER protein chaperone GRP78 in brain cells (Duan and Mattson, 1999; Arumugam et al., 2010).

## VI. Future Directions

### A. Technological Advances

The full extent to which intracellular Ca$^{2+}$ signaling supports cellular functioning is only beginning to be understood, the current knowledge state probably reflecting the tip of a large iceberg. The coming years will certainly provide a new level of understanding, in part because of the concurrent technological advances that can accelerate and target precise Ca$^{2+}$-signaling pathways within cells, as well as within specific organelles. Chemical Ca$^{2+}$ indicators, such as the fura or Oregon Green series of BAPTA-based dyes, have been used for many years and have provided great flexibility in their design and specificity for binding affinity and ratiometric quantitation assays (Paredes et al., 2008). However, the relatively more recent development of genetically encoded Ca$^{2+}$ indicators opens new doors in terms of experimental design and probe specificity. Because these indicators become incorporated at the genome level and are translated as fluorescent proteins that alter their emission intensity as a function of Ca$^{2+}$ levels, they can be used in long-term experiments over days to months as well as introduced into intact animals for in vivo models or used to generate transgenic animals for probing activity over a lifetime (Kotlikoff, 2007; Mank et al., 2008). Although beyond the scale of this review, the optimization of many genetically encoded $Ca^{2+}$ indicators now provides the opportunity to probe $Ca^{2+}$ signaling dynamics in both large and small scales (Rochefort and Konnerth, 2008). Imaging large populations of cells simultaneously allows for detailed insight into network activity (Wilms and Häusser, 2009) in vitro and in vivo, whereas fluorescence resonance energy transfer-based analysis can demonstrate $Ca^{2+}$-dependent responses at the single protein level (Rochefort and Konnerth, 2008). There is a large range of possible applications of these technologies, including single-cell imaging in vivo and the measurement of $Ca^{2+}$ signaling within individual organelles and cellular compartments (Tian et al., 2009). Future developments in this arena will advance a better understanding of the dynamics of intracellular $Ca^{2+}$ signaling.

Another interesting advance involves the modification of the channelrhodopsins, which function as light-activated ion channels in algae, to optically manipulate $Ca^{2+}$ regulation within targeted cells and organelles (Boyden et al., 2005). Genetically redesigned channelrhodopsins can be expressed in a variety of cell types. For example, channelrhodopsin-2, a $Ca^{2+}$-permeable, light-activated ion channel, has been used for triggering $Ca^{2+}$ influx and is particularly useful in excitable cells such as neurons. In this regard, it has been used to as a tool to activate and study synaptic transmission and plasticity. On even broader scales, the behavior of transgenic channelrhodopsin-2-expressing nematodes, fruit flies, zebrafish, and mice has been remote-controlled by optical stimulation. In the future, these types of experimental approaches may serve a variety of new applications and in basic science and translational research studies (Schoenenberger et al., 2011).

### *B. Therapeutic Opportunities*

Because numerous cellular functions rely on intracellular $Ca^{2+}$, dysfunction in ER $Ca^{2+}$ signaling is involved in a range of disease states. Malignant hyperthermia and central core disease are examples of RyR1-mediated neuromuscular diseases, RyR2 mutations play a role in stress-induced polymorphic ventricular tachycardia (a form of cardiac arrhythmia) and arrhythmogenic right ventricular dysplasia, and RyR3 dysfunction may be involved in mood and memory disorders (Mackrill, 2010). As described in section VII, alterations in the expression or function of RyR and $IP_3R$ have been implicated in AD. Altered $IP_3R$ activity has also been linked to cardiac hypertrophy, neurodegenerative diseases, cancer, and metabolic disorders (Stutzmann, 2005; Verkhratsky, 2005; Bezprozvanny and Mattson, 2008; Higazi et al., 2009; Cárdenas et al., 2010). As research progresses, this abbreviated list will probably lengthen considerably. As the awareness of the role of ER $Ca^{2+}$ signaling dysregulation in disease grows, so will targeted therapeutic strategies directed at specific elements of intracellular $Ca^{2+}$ signaling cascades. Some progress in these areas is already under way. For example, dantrolene is a RyR-blocker used in the treatment of malignant hypothermia, as well as neuroleptic malignant syndrome, and muscle spasticity. Other experimental applications for RyR-stabilizing compounds are being tested, such as the benzothiazepine K201 in treatments for heart failure and kidney disease (Mackrill, 2010). Significant hurdles still exist, but these may not be insurmountable. Because of the widespread distribution of the RyR and $IP_3R$, global targeting against an entire receptor class is likely to result in major side-effects; however, the anticipated development of reagents against specific receptor subtypes will better target and address disease states. With the confluence of technological advances under development to probe intracellular $Ca^{2+}$, and the growing knowledge of the functions and diseases in which ER $Ca^{2+}$ is involved, the coming years will probably bring a surge of new information and therapeutic strategies targeting ER $Ca^{2+}$ pathways.

#### Acknowledgments

This work was supported in part by the Intramural Research Program of the National Institutes of Health National Institute on Aging (to M.M.); and by the National Institutes of Health National Institute on Aging [Grant AG030205] (to G.E.S.). We thank K. C. Alexander for assistance in the preparation of figures and Corinne Schneider for editorial assistance.

#### Authorship Contributions

*Wrote or contributed to the writing of the manuscript:* Stutzmann and Mattson.

#### References

Alford S, Frenguelli BG, Schofield JG, and Collingridge GL (1993) Characterization of $Ca^{2+}$ signals induced in hippocampal CA1 neurones by the synaptic activation of NMDA receptors. *J Physiol* **469:**693–716.

Allbritton NL, Meyer T, and Stryer L (1992) Range of messenger action of calcium ion and inositol 1,4,5-trisphosphate. *Science* **258:**1812–1815.

Alvarez J and Montero M (2002) Measuring $[Ca^{2+}]$ in the endoplasmic reticulum with aequorin. *Cell Calcium* **32:**251–260.

Andersen JP and Vilsen B (1998) Structure-function relationships of the calcium binding sites of the sarcoplasmic reticulum $Ca^{2+}$-ATPase. *Acta Physiol Scand Suppl* **643:**45–54.

Andersson KB, Birkeland JA, Finsen AV, Louch WE, Sjaastad I, Wang Y, Chen J, Molkentin JD, Chien KR, Sejersted OM, et al. (2009) Moderate heart dysfunction in mice with inducible cardiomyocyte-specific excision of the Serca2 gene. *J Mol Cell Cardiol* **47:**180–187.

Arfi A, Zisling R, Richard E, Batista L, Poenaru L, Futerman AH, and Caillaud C (2006) Reversion of the biochemical defects in murine embryonic Sandhoff neurons using a bicistronic lentiviral vector encoding hexosaminidase alpha and beta. *J Neurochem* **96:**1572–1579.

Aridor M and Fish KN (2009) Selective targeting of ER exit sites supports axon development. *Traffic* **10:**1669–1684.

Aridor M, Guzik AK, Bielli A, and Fish KN (2004) Endoplasmic reticulum export site formation and function in dendrites. *J Neurosci* **24:**3770–3776.

Arispe N, Rojas E, and Pollard HB (1993) Alzheimer disease amyloid beta protein forms calcium channels in bilayer membranes: blockade by tromethamine and aluminum. *Proc Natl Acad Sci USA* **90:**567–571.

Arredouani A (2004) Diversification of function and pharmacology in intracellular $Ca^{2+}$ signalling. *Cell Science Rev* **1:**30–79.

Arumugam TV, Phillips TM, Cheng A, Morrell CH, Mattson MP, and Wan R (2010) Age and energy intake interact to modify cell stress pathways and stroke outcome. *Ann Neurol* **67:**41–52.

Auffret A, Gautheron V, Mattson MP, Mariani J, and Rovira C (2010) Progressive age-related impairment of the late long-term potentiation in Alzheimer's disease presenilin-1 mutant knock-in mice. *J Alzheimers Dis* **19:**1021–1033.

Avila J, Pérez M, Lim F, Gómez-Ramos A, Hernández F, and Lucas JJ (2004) Tau in neurodegenerative diseases: tau phosphorylation and assembly. *Neurotox Res* **6:**477–482.

Baker KD, Edwards TM, and Rickard NS (2008) Inhibition of mGluR1 and IP3Rs impairs long-term memory formation in young chicks. *Neurobiol Learn Mem* **90:**269–274.

Bardo S, Cavazzini MG, and Emptage N (2006) The role of endoplasmic reticulum Ca$^{2+}$ store in the plasticity of central neurons. *Trends Pharmacol Sci* **27:**78–84.

Beard NA, Laver DR, and Dulhunty AF (2004) Calsequestrin and the calcium release channel of skeletal and cardiac muscle. *Prog Biophys Mol Biol* **85:**33–69.

Beecham GW, Schnetz-Boutaud N, Haines JL, and Pericak-Vance MA (2009) CALHM1 polymorphism is not associated with late-onset Alzheimer disease. *Ann Hum Genet* **73:**379–381.

Bendayan M (1989) Ultrastructural localization of insulin and C-peptide antigenic sites in rat pancreatic B cell obtained by applying the quantitative high-resolution protein A-gold approach. *Am J Anat* **185:**205–216.

Bergamaschini L, Rossi E, Storini C, Pizzimenti S, Distaso M, Perego C, De Luigi A, Vergani C, and De Simoni MG (2004) Peripheral treatment with enoxaparin, a low molecular weight heparin, reduces plaques and beta-amyloid accumulation in a mouse model of Alzheimer's disease. *J Neurosci* **24:**4181–4186.

Berna-Erro A, Braun A, Kraft R, Kleinschnitz C, Schuhmann MK, Stegner D, Wultsch T, Eilers J, Meuth SG, Stoll G, et al. (2009) STIM2 regulates capacitive Ca$^{2+}$ entry in neurons and plays a key role in hypoxic neuronal cell death. *Sci Signal* **2:**ra67.

Berridge MJ (1997) Elementary and global aspects of calcium signalling. *J Physiol* **499:**291–306.

Berridge MJ (1998) Neuronal calcium signaling. *Neuron* **21:**13–26.

Berridge MJ (2002) The endoplasmic reticulum: a multifunctional signaling organelle. *Cell Calcium* **32:**235–249.

Bertram L, Schjeide BM, Hooli B, Mullin K, Hiltunen M, Soininen H, Ingelsson M, Lannfelt L, Blacker D, and Tanzi RE (2008) No association between CALHM1 and Alzheimer's disease risk. *Cell* **135:**993–994.

Best L, Brown PD, Sener A, and Malaisse WJ (2010) Electrical activity in pancreatic islet cells: the VRAC hypothesis. *Islets* **2:**59–64.

Betzenhauser MJ and Marks AR (2010) Ryanodine receptor channelopathies. *Pflugers Arch* **460:** 467–480.

Bezprozvanny I (2005) The inositol 1,4,5-trisphosphate receptors. *Cell Calcium* 38: 261-272.

Bezprozvanny I and Mattson MP (2008) Neuronal Ca$^{2+}$ mishandling and the pathogenesis of Alzheimer's disease. *Trends Neurosci* **15:**454–463.

Bickler PE, Fahlman CS, Gray J, and McKleroy W (2009) Inositol 1,4,5-triphosphate receptors and NAD(P)H mediate Ca$^{2+}$ signaling required for hypoxic preconditioning of hippocampal neurons. *Neuroscience* **160:**51–60.

Black VH, Sanjay A, van Leyen K, Lauring B, and Kreibich G (2005) Cholesterol and steroid synthesizing smooth endoplasmic reticulum of adrenocortical cells contains high levels of proteins associated with the translocation channel. *Endocrinology* **146:**4234–4249.

Boada M, Antúnez C, López-Arrieta J, Galán JJ, Morón FJ, Hernández I, Marín J, Martínez-Lage P, Alegret M, Carrasco JM, et al. (2010) CALHM1 P86L polymorphism is associated with late-onset Alzheimer's disease in a recessive model. *J Alzheimers Dis* **20:**247–251.

Boehning D, Mak DO, Foskett JK, and Joseph SK (2001) Molecular determinants of ion permeation and selectivity in inositol 1,4,5-trisphosphate receptor Ca$^{2+}$ channels. *J Biol Chem* **276:**13509–13512.

Boehning D, Patterson RL, Sedaghat L, Glebova NO, Kurosaki T, and Snyder SH (2003) Cytochrome c binds to inositol (1,4,5) trisphosphate receptors, amplifying calcium-dependent apoptosis. *Nat Cell Biol* **5:**1051–1061.

Bogdanov KY, Vinogradova TM, and Lakatta EG (2001) Sinoatrial nodal cell ryanodine receptor and Na$^{+}$-Ca$^{2+}$ exchanger: molecular partners in pacemaker regulation. *Circ Res* **88:**1254–1258.

Bola B and Allan V (2009) How and why does the endoplasmic reticulum move? *Biochem Soc Trans* **37:**961–965.

Boncompagni S and Protasi F (2007) Tethers: structural connections between SR and the outer mitochondria membrane. *Biophys J* **92:**A313.

Bootman MD, Collins TJ, Mackenzie L, Roderick HL, Berridge MJ, and Peppiatt CM (2002) 2-aminoethoxydiphenyl borate (2-APB) is a reliable blocker of store-operated Ca$^{2+}$ entry but an inconsistent inhibitor of InsP3-induced Ca$^{2+}$ release. *FASEB J* **16:**1145–1150.

Bouchard R, Pattarini R, and Geiger JD (2003) Presence and functional significance of presynaptic ryanodine receptors. *Prog Neurobiol* **69:**391–418.

Boyden ES, Zhang F, Bamberg E, Nagel G, and Deisseroth K (2005) Millisecond-timescale, genetically targeted optical control of neural activity. *Nature Neuroscience* **8:**1263–1268.

Boys JA, Toledo AH, Anaya-Prado R, Lopez-Neblina F, and Toledo-Pereyra LH (2010) Effects of dantrolene on ischemia-reperfusion injury in animal models: a review of outcomes in heart, brain, liver, and kidney. *J Investig Med* **58:**875–882.

Bruce-Keller AJ, Begley JG, Fu W, Butterfield DA, Bredesen DE, Hutchins JB, Hensley K, and Mattson MP (1998) Bcl-2 protects isolated plasma and mitochondrial membranes against lipid peroxidation induced by hydrogen peroxide and amyloid beta-peptide. *J Neurochem* **70:**31–39.

Bruce-Keller AJ, Umberger G, McFall R, and Mattson MP (1999) Food restriction reduces brain damage and improves behavioral outcome following excitotoxic and metabolic insults. *Ann Neurol* **45:**8–15.

Buckingham SD, Jones AK, Brown LA, and Sattelle DB (2009) Nicotinic acetylcholine receptor signalling: roles in Alzheimer's disease and amyloid neuroprotection. *Pharmacol Rev* **61:**39–61.

Bull R, Finkelstein JP, Gálvez J, Sánchez G, Donoso P, Behrens MI, and Hidalgo C (2008) Ischemia enhances activation by Ca$^{2+}$ and redox modification of ryanodine receptor channels from rat brain cortex. *J Neurosci* **28:**9463–9472.

Burdakov D, Petersen OH, and Verkhratsky A (2005) Intraluminal calcium as a primary regulator of endoplasmic reticulum function. *Cell Calcium* **38:**303–310.

Burdakov D and Verkhratsky A (2006) Biophysical re-equilibration of Ca$^{2+}$ fluxes as a simple biologically plausible explanation for complex intracellular Ca$^{2+}$ release patterns. *FEBS Lett* **580:**463–468.

Butanda-Ochoa A, Höjer G, Morales-Tlalpan V, and Díaz-Muñoz M (2006) Recognition and activation of ryanodine receptors by purines. *Curr Med Chem* **13:**647–657.

Bygrave FL and Benedetti A (1996) What is the concentration of calcium ions in the endoplasmic reticulum? *Cell Calcium* **19:**547–551.

Cahalan MD (2009) STIMulating store-operated Ca$^{2+}$ entry. *Nat Cell Biol* **11:**669–677.

Calabrese EJ, Bachmann KA, Bailer AJ, Bolger PM, Borak J, Cai L, Cedergreen N, Cherian MG, Chiueh CC, Clarkson TW, et al. (2007) Biological stress response terminology: integrating the concepts of adaptive response and preconditioning stress within a hormetic dose-response framework. *Toxicol Appl Pharmacol* **222:** 122–128.

Camandola S and Mattson MP (2011) Aberrant subcellular neuronal calcium regulation in aging and Alzheimer's disease. *Biochim Biophys Acta* **1813:**965–973.

Camello C, Lomax R, Petersen OH, and Tepikin AV (2002) Calcium leak from intracellular stores–the enigma of calcium signaling. *Cell Calcium* **32:**355–361.

Carafoli E and Brini M (2000) Calcium pumps: structural basis for and mechanism of calcium transmembrane transport. *Curr Opin Chem Biol* **4:**152–161.

Cárdenas C, Miller RA, Smith I, Bui T, Molgó J, Müller M, Vais H, Cheung KH, Yang J, Parker I, et al. (2010) Essential regulation of cell bioenergetics by constitutive InsP3 receptor Ca$^{2+}$ transfer to mitochondria. *Cell* **142:**270–283.

Carrasco MA, Jaimovich E, Kemmerling U, and Hidalgo C (2004) Signal transduction and gene expression regulated by calcium release from internal stores in excitable cells. *Biol Res* **37:**701–712.

Casley CS, Lakics V, Lee HG, Broad LM, Day TA, Cluett T, Smith MA, O'Neill MJ, and Kingston AE (2009) Up-regulation of astrocyte metabotropic glutamate receptor 5 by amyloid-beta peptide. *Brain Res* **1260:**65–75.

Catterall WA, Perez-Reyes E, Snutch TP, and Striessnig J (2005) International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. *Pharmacol Rev* **57:**411–425.

Chakroborty S, Goussakov I, Miller MB, and Stutzmann GE (2009) Deviant ryanodine receptor-mediated calcium release resets synaptic homeostasis in presymptomatic 3xTg-AD mice. *J Neurosci* **29:**9458–9470.

Chan CS, Gertler TS, and Surmeier DJ (2009) Calcium homeostasis, selective vulnerability and Parkinson's disease. *Trends Neurosci* **32:**249–256.

Chan SL, Liu D, Kyriazis GA, Bagsiyao P, Ouyang X, and Mattson MP (2006) Mitochondrial uncoupling protein-4 regulates calcium homeostasis and sensitivity to store depletion-induced apoptosis in neural cells. *J Biol Chem* **281:**37391–37403.

Chan SL, Mayne M, Holden CP, Geiger JD, and Mattson MP (2000) Presenilin-1 mutations increase levels of ryanodine receptors and calcium release in PC12 cells and cortical neurons. *J Biol Chem* **275:**18195–18200.

Chen FW, Li C, and Ioannou YA (2010) Cyclodextrin induces calcium-dependent lysosomal exocytosis. *PLoS One* **5:**e15054.

Cheung KH, Mei L, Mak DO, Hayashi I, Iwatsubo T, Kang DE, and Foskett JK (2010) Gain-of-function enhancement of IP3 receptor modal gating by familial Alzheimer's disease-linked presenilin mutants in human cells and mouse neurons. *Sci Signal* **3:**ra22–ra30.

Cheung KH, Shineman D, Müller M, Cárdenas C, Mei L, Yang J, Tomita T, Iwatsubo T, Lee VM, and Foskett JK (2008) Mechanism of Ca$^{2+}$ disruption in Alzheimer's disease by presenilin regulation of InsP$_3$ receptor channel gating. *Neuron* **58:**871–883.

Chigurupati S, Mughal MR, Chan SL, Arumugam TV, Baharani A, Tang SC, Yu QS, Holloway HW, Wheeler R, Poosala S, et al. (2010) A synthetic uric acid analog accelerates cutaneous wound healing in mice. *PLoS One* **5:**e10044.

Chigurupati S, Wei Z, Belal C, Vandermey M, Kyriazis GA, Arumugam TV, and Chan SL (2009) The homocysteine-inducible endoplasmic reticulum stress protein counteracts calcium store depletion and induction of CCAAT enhancer-binding protein homologous protein in a neurotoxin model of Parkinson disease. *J Biol Chem* **284:**18323–18333.

Cribbs DH, Pike CJ, Weinstein SL, Velazquez P, and Cotman CW (1997) All-D-enantiomers of beta-amyloid exhibit similar biological properties to all-L-beta-amyloids. *J Biol Chem* **272:**7431–7436.

Cui PJ, Zheng L, Cao L, Wang Y, Deng YL, Wang G, Xu W, Tang HD, Ma JF, Zhang T, et al. (2010) CALHM1 P86L polymorphism is a risk factor for Alzheimer's disease in the Chinese population. *J Alzheimers Dis* **19:**31–35.

Cutler RG, Kelly J, Storie K, Pedersen WA, Tammara A, Hatanpaa K, Troncoso JC, and Mattson MP (2004) Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease. *Proc Natl Acad Sci USA* **101:**2070–2075.

Darby PJ, Kwan CY, and Daniel EE (1993) Use of calcium pump inhibitors in the study of calcium regulation in smooth muscle. *Biol Signals* **2:**293–304.

Dawson TM, Ko HS, and Dawson VL (2010) Genetic animal models of Parkinson's disease. *Neuron* **66:**646–661.

del Monte F, Harding SE, Schmidt U, Matsui T, Kang ZB, Dec GW, Gwathmey JK, Rosenzweig A, and Hajjar RJ (1999) Restoration of contractile function in isolated cardiomyocytes from failing human hearts by gene transfer of SERCA2a. *Circulation* **100:**2308–2311.

del Monte F, Lebeche D, Guerrero JL, Tsuji T, Doye AA, Gwathmey JK, and Hajjar RJ (2004) Abrogation of ventricular arrhythmias in a model of ischemia and reperfusion by targeting myocardial calcium cycling. *Proc Natl Acad Sci* **101:**5622–5627.

Demuro A, Parker I, and Stutzmann GE (2010) Calcium signaling and amyloid toxicity in Alzheimer disease. *J Biol Chem* **285:**12463–12468.

Denborough M (1998) Malignant hyperthermia. *Lancet* **352:**1131–1136.

Denmeade SR and Isaacs JT (2005) The SERCA pump as a therapeutic target: making a "smart bomb" for prostate cancer. *Cancer Biol Ther* **4:**14–22.

DiBernardo AB and Cudkowicz ME (2006) Translating preclinical insights into effective human trials in ALS. *Biochim Biophys Acta* **1762:**1139–1149.

Dietrich J, Jourdon P, and Diacono J (1976) [The effect of caffeine on the electrical and mechanical activities of isolated rat and guinea pig hearts: discussion of the respective roles of calcium mobility and catecholamines in the two species]. *Therapie* **31:**525–539.

Dode L, De Greef C, Mountian I, Attard M, Town MM, Casteels R, and Wuytack F (1998) Structure of the human sarco/endoplasmic reticulum Ca$^{2+}$-ATPase 3 gene. Promoter analysis and alternative splicing of the SERCA3 pre-mRNA. *J Biol Chem* **273:**13982–13994.

Donoso P, Prieto H, and Hidalgo C (1995) Luminal calcium regulates calcium release in triads isolated from frog and rabbit skeletal muscle. *Biophys J* **68:**507–515.

Dreses-Werringloer U, Lambert JC, Vingtdeux V, Zhao H, Vais H, Siebert A, Jain A, Koppel J, Rovelet-Lecrux A, Hannequin D, et al. (2008) A polymorphism in CALHM1 influences Ca$^{2+}$ homeostasis, A$\beta$ levels, and Alzheimer's disease risk. *Cell* **133:**1149–1161.

Duan W and Mattson MP (1999) Dietary restriction and 2-deoxyglucose administration improve behavioral outcome and reduce degeneration of dopaminergic neurons in models of Parkinson's disease. *J Neurosci Res* **57:**195–206.

Duchen MR (1999) Contributions of mitochondria to animal physiology: from homeostatic sensor to calcium signalling and cell death. *J Physiol* **516:**1–17.

Eckenrode EF, Yang J, Velmurugan GV, Foskett JK, and White C (2010) Apoptosis protection by Mcl-1 and Bcl-2 modulation of inositol 1,4,5-trisphosphate receptor-dependent Ca$^{2+}$ signaling. *J Biol Chem* **285:**13678–13684.

Emptage N, Bliss TV, and Fine A (1999) Single synaptic events evoke NMDA receptor-mediated release of calcium from internal stores in hippocampal dendritic spines. *Neuron* **22:**115–124.

Emptage NJ, Reid CA, and Fine A (2001) Calcium stores in hippocampal synaptic boutons mediate short-term plasticity, store-operated Ca$^{2+}$ entry, and spontaneous transmitter release. *Neuron* **29:**197–208.

Engelhardt JI, Siklós L, Kömüves L, Smith RG, and Appel SH (1995) Antibodies to calcium channels from ALS patients passively transferred to mice selectively increase intracellular calcium and induce ultrastructural changes in motoneurons. *Synapse* **20:**185–199.

Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N, and van Duijn CM (1997) Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA* **278:**1349–1356.

Fein A (2003) Inositol 1,4,5-trisphosphate-induced calcium release is necessary for generating the entire light response of limulus ventral photoreceptors. *J Gen Physiol* **121:**441–449.

Fernyhough P and Calcutt NA (2010) Abnormal calcium homeostasis in peripheral neuropathies. *Cell Calcium* **47:**130–139.

Fill M and Copello JA (2002) Ryanodine receptor calcium release channels. *Physiol Rev* **82:**893–922.

Finch EA, Turner TJ, and Goldin SM (1991) Ca$^{2+}$ as a coagonist of inositol-1,4,5-trisphosphate-induced calcium release. *Science* **252:**443–446.

Fitzjohn SM and Collingridge GL (2002) Calcium stores and synaptic plasticity. *Cell Calcium* **32:**405–411.

Flourakis M, Van Coppenolle F, Lehen'kyi V, Beck B, Skryma R, and Prevarskaya N (2006) Passive calcium leak via translocon is a first step for iPLA2-pathway regulated store operated channels activation. *FASEB J* **20:**1215–1217.

Foskett JK, White C, Cheung KH, and Mak DO (2007) Inositol trisphosphate receptor Ca$^{2+}$ release channels. *Physiol Rev* **87:**593–658.

Franzini-Armstrong C (2007) ER-mitochondria communication. How privileged? *Physiology* **22:**261–268.

Friel DD and Tsien RW (1992) A caffeine- and ryanodine-sensitive Ca$^{2+}$ store in bullfrog sympathetic neurones modulates effects of Ca$^{2+}$ entry on [Ca$^{2+}$]$_i$. *J Physiol* **450:**217–246.

Fujii S, Matsumoto M, Igarashi K, Kato H, and Mikoshiba K (2000) Synaptic plasticity in hippocampal CA1 neurons of mice lacking type 1 inositol-1,4,5-triphosphate receptors. *Learn Mem* **7:**312–320.

Furuichi T, Furutama D, Hakamata Y, Nakai J, Takeshima H, and Mikoshiba K (1994) Multiple types of ryanodine receptor/Ca$^{2+}$ release channels are differentially expressed in rabbit brain. *J Neurosci* **14:**4794–4805.

Furukawa K and Mattson MP (1998) The transcription factor NF-kappaB mediates increases in calcium currents and decreases in NMDA- and AMPA/kainate-induced currents induced by tumor necrosis factor-alpha in hippocampal neurons. *J Neurochem* **70:**1876–1886.

Futatsugi A, Kato K, Ogura H, Li ST, Nagata E, Kuwajima G, Tanaka K, Itohara S, and Mikoshiba K (1999) Facilitation of NMDAR-independent LTP and spatial learning in mutant mice lacking ryanodine receptor type 3. *Neuron* **24:**701–713.

Gafni J, Munsch JA, Lam TH, Catlin MC, Costa LG, Molinski TF, and Pessah IN (1997) Xestospongins: potent membrane permeable blockers of the inositol 1,4,5-trisphosphate receptor. *Neuron* **19:**723–733.

George CH, Higgs GV, and Lai FA (2003) Ryanodine receptor mutations associated with stress-induced ventricular tachycardia mediate increased calcium release in stimulated cardiomyocytes. *Circ Res* **93:**531–540.

Gerasimenko JV, Flowerdew SE, Voronina SG, Sukhomlin TK, Tepikin AV, Petersen OH, and Gerasimenko OV (2006) Bile acids induce Ca$^{2+}$ release from both the endoplasmic reticulum and acidic intracellular calcium stores through activation of inositol trisphosphate receptors and ryanodine receptors. *J Biol Chem* **281:**40154–40163.

Ghassemi F, Vukcevic M, Xu L, Zhou H, Meissner G, Muntoni F, Jungbluth H, Zorzato F, and Treves S (2009) A recessive ryanodine receptor 1 mutation in a CCD patient increases channel activity. *Cell Calcium* **45:**192–197.

Gilon P, Arredouani A, Gailly P, Gromada J, and Henquin JC (1999) Uptake and release of Ca$^{2+}$ by the endoplasmic reticulum contribute to the oscillations of the cytosolic Ca$^{2+}$ concentration triggered by Ca$^{2+}$ influx in the electrically excitable pancreatic B-cell. *J Biol Chem* **274:**20197–20205.

Glazner GW, Camandola S, Geiger JD, and Mattson MP (2001) Endoplasmic reticulum D-myo-inositol 1,4,5-trisphosphate-sensitive stores regulate nuclear factor-kappaB binding activity in a calcium-independent manner. *J Biol Chem* **276:**22461–22467.

Goldman WP, Price JL, Storandt M, Grant EA, McKeel DW Jr, Rubin EH, and Morris JC (2001) Absence of cognitive impairment or decline in preclinical Alzheimer's disease. *Neurology* **56:**361–367.

Gómez-Ramos A, Díaz-Hernández M, Cuadros R, Hernández F, and Avila J (2006) Extracellular tau is toxic to neuronal cells. *FEBS Lett* **580:**4842–4850.

Goussakov I, Miller MB, and Stutzmann GE (2010) NMDA-mediated Ca$^{2+}$ influx drives ryanodine receptor activation in dendrites of young Alzheimer's disease mice. *J Neurosci* **30:**12128–12137.

Griffing LR (2010) Networking in the endoplasmic reticulum. *Biochem Soc Trans* **38:**747–753.

Grigoriev I, Gouveia SM, van der Vaart B, Demmers J, Smyth JT, Honnappa S, Splinter D, Steinmetz MO, Putney JW Jr, Hoogenraad CC, et al. (2008) STIM1 is a MT-plus-end-tracking protein involved in remodeling of the ER. *Curr Biol* **18:**177–182.

Grosskreutz J, Van Den Bosch L, and Keller BU (2010) Calcium dysregulation in amyotrophic lateral sclerosis. *Cell Calcium* **47:**165–174.

Guis S, Figarella-Branger D, Monnier N, Bendahan D, Kozak-Ribbens G, Mattei JP, Lunardi J, Cozzone PJ, and Pellissier JF (2004) Multiminicore disease in a family susceptible to malignant hyperthermia: histology, in vitro contracture tests, and genetic characterization. *Arch Neurol* **61:**106–113.

Guo Q, Fu W, Sopher BL, Miller MW, Ware CB, Martin GM, and Mattson MP (1999b) Increased vulnerability of hippocampal neurons to excitotoxic necrosis in presenilin-1 mutant knock-in mice. *Nat Med* **5:**101–106.

Guo Q, Furukawa K, Sopher BL, Pham DG, Xie J, Robinson N, Martin GM, and Mattson MP (1996) Alzheimer's PS-1 mutation perturbs calcium homeostasis and sensitizes PC12 cells to death induced by amyloid beta-peptide. *Neuroreport* **8:**379–383.

Guo Q, Sebastian L, Sopher BL, Miller MW, Ware CB, Martin GM, and Mattson MP (1999a) Increased vulnerability of hippocampal neurons from presenilin-1 mutant knock-in mice to amyloid beta-peptide toxicity: central roles of superoxide production and caspase activation. *J Neurochem* **72:**1019–1029.

Guo Q, Sopher BL, Furukawa K, Pham DG, Robinson N, Martin GM, and Mattson MP (1997) Alzheimer's presenilin mutation sensitizes neural cells to apoptosis induced by trophic factor withdrawal and amyloid beta-peptide: involvement of Ca$^{2+}$ and oxyradicals. *J Neurosci* **17:**4212–4222.

Guo Z, Kindy MS, Kruman I, and Mattson MP (2000) ALS-linked Cu/Zn-SOD mutation impairs cerebral synaptic glucose and glutamate transport and exacerbates ischemic brain injury. *J Cereb Blood Flow Metab* **20:**463–468.

Gwak M, Park P, Kim K, Lim K, Jeong S, Baek C, and Lee J (2008) The effects of dantrolene on hypoxic-ischemic injury in the neonatal rat brain. *Anesth Analg* **106:**227–233.

Györke I and Györke S (1998) Regulation of the cardiac ryanodine receptor channel by luminal Ca$^{2+}$ involves luminal Ca$^{2+}$ sensing sites. *Biophysical Journal* **75:**2801–2810.

Györke I, Hester N, Jones LR, and Györke S (2004) The role of calsequestrin, triadin, and junctin in conferring cardiac ryanodine receptor responsiveness to luminal calcium. *Biophys J* **86:**2121–2128.

Haberman F, Tang SC, Arumugam TV, Hyun DH, Yu QS, Cutler RG, Guo Z, Holloway HW, Greig NH, and Mattson MP (2007) Soluble neuroprotective antioxidant uric acid analogs ameliorate ischemic brain injury in mice. *Neuromolecular Med* **9:**315–323.

Hagenston AM, Fitzpatrick JS, and Yeckel MF (2008) MGluR-mediated calcium waves that invade the soma regulate firing in layer V medial prefrontal cortical pyramidal neurons. *Cereb Cortex* **18:**407–423.

Halagappa VK, Guo Z, Pearson M, Matsuoka Y, Cutler RG, Laferla FM, and Mattson MP (2007) Intermittent fasting and caloric restriction ameliorate age-related behavioral deficits in the triple-transgenic mouse model of Alzheimer's disease. *Neurobiol Dis* **26:**212–220.

Harris KM (1999) Structure, development, and plasticity of dendritic spines. *Curr Opin Neurobiol* **9:**343–348.

Harvey J and Collingridge GL (1992) Thapsigargin blocks the induction of long-term potentiation in rat hippocampal slices. *Neurosci Lett* **139:**197–200.

Hata T, Noda T, Nishimura M, and Watanabe Y (1996) The role of Ca$^{2+}$ release from sarcoplasmic reticulum in the regulation of sinoatrial node automaticity. *Heart Vessels* **11:**234–241.

Hernández-Fonseca K and Massieu L (2005) Disruption of endoplasmic reticulum calcium stores is involved in neuronal death induced by glycolysis inhibition in cultured hippocampal neurons. *J Neurosci Res* **82:**196–205.

Hertle DN and Yeckel MF (2007) Distribution of inositol-1,4,5-trisphosphate receptor isotypes and ryanodine receptor isotypes during maturation of the rat hippocampus. *Neuroscience* **150:**625–638.

Hewavitharana T, Deng X, Soboloff J, and Gill DL (2007) Role of STIM and Orai proteins in the store-operated calcium signaling pathway. *Cell Calcium* **42:**173–182.

Higazi DR, Fearnley CJ, Drawnel FM, Talasila A, Corps EM, Ritter O, McDonald F, Mikoshiba K, Bootman MD, and Roderick HL (2009) Endothelin-1-stimulated InsP3-induced Ca$^{2+}$ release is a nexus for hypertrophic signaling in cardiac myocytes. *Mol Cell* **33:**472–482.

Holbro N, Grunditz A, and Oertner TG (2009) Differential distribution of endoplasmic reticulum controls metabotropic signaling and plasticity at hippocampal synapses. *Proc Natl Acad Sci USA* **106:**15055–15060.

Hoover BR, Reed MN, Su J, Penrod RD, Kotilinek LA, Grant MK, Pitstick R, Carlson GA, Lanier LM, Yuan LL, et al. (2010) Tau mislocalization to dendritic spines mediates synaptic dysfunction independently of neurodegeneration. *Neuron* **68:**1067–1081.

Huang TJ, Sayers NM, Fernyhough P, and Verkhratsky A (2002) Diabetes-induced alterations in calcium homeostasis in sensory neurones of streptozotocin-diabetic rats are restricted to lumbar ganglia and are prevented by neurotrophin-3. *Diabetologia* **45:**560–570.

Inoue M, Sakamoto Y, Fujishiro N, Imanaga I, Ozaki S, Prestwich GD, and

Warashina A (2003) Homogeneous $Ca^{2+}$ stores in rat adrenal chromaffin cells. *Cell Calcium* **33:**19–26.

Inoue S (2008) In situ Aβ pores in AD brain are cylindrical assembly of Aβ protofilaments. *Amyloid* **15:**223–233.

Iqbal K, Alonso Adel C, Chen S, Chohan MO, El-Akkad E, Gong CX, Khatoon S, Li B, Liu F, Rahman A, et al. (2005) Tau pathology in Alzheimer disease and other tauopathies. *Biochim Biophys Acta* **1739:**198–210.

Irizarry MC, Raman R, Schwarzschild MA, Becerra LM, Thomas RG, Peterson RC, Ascherio A, and Aisen PS (2009) Plasma urate and progression of mild cognitive impairment. *Neurodegener Dis* **6:**23–28.

Isaacs AM, Senn DB, Yuan M, Shine JP, and Yankner BA (2006) Acceleration of amyloid beta-peptide aggregation by physiological concentrations of calcium. *J Biol Chem* **281:**27916–27923.

Ito E, Oka K, Etcheberrigaray R, Nelson TJ, McPhie DL, Tofel-Grehl B, Gibson GE, and Alkon DL (1994) Internal $Ca^{2+}$ mobilization is altered in fibroblasts from patients with Alzheimer disease. *Proc Natl Acad Sci* **91:**534–538.

Jahanshahi P, Wu R, Carter JD, and Nunemaker CS (2009) Evidence of diminished glucose stimulation and endoplasmic reticulum function in nonoscillatory pancreatic islets. *Endocrinology* **150:**607–615.

James G and Butt AM (2002) P2Y and P2X purinoceptor mediated $Ca^{2+}$ signalling in glial cell pathology in the central nervous system. *Eur J Pharmacol* **447:**247–260.

Janczewski AM and Lakatta EG (2010) Modulation of sarcoplasmic reticulum $Ca^{2+}$ cycling in systolic and diastolic heart failure associated with aging. *Heart Fail Rev* **15:**431–445.

Jiang D, Wang R, Xiao B, Kong H, Hunt DJ, Choi P, Zhang L, and Chen SR (2005) Enhanced store overload-induced $Ca^{2+}$ release and channel sensitivity to luminal $Ca^{2+}$ activation are common defects of RyR2 mutations linked to ventricular tachycardia and sudden death. *Circ Res* **97:**1173–1181.

Jungbluth H, Müller CR, Halliger-Keller B, Brockington M, Brown SC, Feng L, Chattopadhyay A, Mercuri E, Manzur AY, Ferreiro A, et al. (2002) Autosomal recessive inheritance of RYR1 mutations in a congenital myopathy with cores. *Neurology* **59:**284–287.

Kapogiannis D and Mattson MP (2011) Disrupted energy metabolism and neuronal circuit dysfunction in cognitive impairment and Alzheimer's disease. *Lancet Neurol* **10:**187–198.

Kawase Y and Hajjar RJ (2008) The cardiac sarcoplasmic/endoplasmic reticulum calcium ATPase: a potent target for cardiovascular diseases. *Nat Clin Pract Cardiovasc Med* **5:**554–565.

Keil JM, Shen Z, Briggs SP, and Patrick GN (2010) Regulation of STIM1 and SOCE by the ubiquitin-proteasome system (UPS). *PLoS One* **5:**e13465.

Keller JN, Guo Q, Holtsberg FW, Bruce-Keller AJ, and Mattson MP (1998) Increased sensitivity to mitochondrial toxin-induced apoptosis in neural cells expressing mutant presenilin-1 is linked to perturbed calcium homeostasis and enhanced oxyradical production. *J Neurosci* **18:**4439–4450.

Kelly JF, Furukawa K, Barger SW, Rengen MR, Mark RJ, Blanc EM, Roth GS, and Mattson MP (1996) Amyloid beta-peptide disrupts carbachol-induced muscarinic cholinergic signal transduction in cortical neurons. *Proc Natl Acad Sci USA* **93:**6753–6758.

Kittner B, Rössner M, and Rother M (1997) Clinical trials in dementia with propentofylline. *Ann NY Acad Sci* **826:**307–316.

Knollmann BC (2009) New roles of calsequestrin and triadin in cardiac muscle. *J Physiol* **587:**3081–3087.

Koizumi S, Lipp P, Berridge MJ, and Bootman MD (1999) Regulation of ryanodine receptor opening by lumenal $Ca^{2+}$ underlies quantal $Ca^{2+}$ release in PC12 cells. *J Biol Chem* **274:**33327–33333.

Kolter T and Sandhoff K (2005) Principles of lysosomal membrane digestion: stimulation of sphingolipid degradation by sphingolipid activator proteins and anionic lysosomal lipids. *Annu Rev Cell Dev Biol* **21:**81–103.

Korkotian E, Schwarz A, Pelled D, Schwarzmann G, Segal M, and Futerman AH (1999) Elevation of intracellular glucosylceramide levels results in an increase in endoplasmic reticulum density and in functional calcium stores in cultured neurons. *J Biol Chem* **274:**21673–21678.

Kotlikoff MI (2007) Genetically encoded $Ca^{2+}$ indicators: using genetics and molecular design to understand complex physiology. *J Physiol* **578:**55–67.

Kruglikov I, Gryshchenko O, Shutov L, Kostyuk E, Kostyuk P, and Voitenko N (2004) Diabetes-induced abnormalities in ER calcium mobilization in primary and secondary nociceptive neurons. *Pflugers Arch* **448:**395–401.

Kruman II, Pedersen WA, Springer JE, and Mattson MP (1999) ALS-linked Cu/Zn-SOD mutation increases vulnerability of motor neurons to excitotoxicity by a mechanism involving increased oxidative stress and perturbed calcium homeostasis. *Exp Neurol* **160:**28–39.

Kuromi H and Kidokoro Y (2002) Selective replenishment of two vesicle pools depends on the source of $Ca^{2+}$ at the Drosophila synapse. *Neuron* **35:**333–343.

LaFerla FM (2002) $Ca^{2+}$ dyshomeostasis and intracellular signaling in Alzheimer's disease. *Nat Rev Neurosci* **3:**862–872.

Lahat H, Pras E, Olender T, Avidan N, Ben-Asher E, Man O, Levy-Nissenbaum E, Khoury A, Lorber A, Goldman B, et al. (2001) A missense mutation in a highly conserved region of CASQ2 is associated with autosomal recessive catecholamine-induced polymorphic ventricular tachycardia in Bedouin families from Israel. *Am J Hum Genet* **69:**1378–1384.

Lam M, Dubyak G, Chen L, Nuñez G, Miesfeld RL, and Distelhorst CW (1994) Evidence that BCL-2 represses apoptosis by regulating endoplasmic reticulum-associated $Ca^{2+}$ fluxes. *Proc Natl Acad Sci USA* **91:**6569–6573.

Lambert JC, Sleegers K, González-Pérez A, Ingelsson M, Beecham GW, Hiltunen M, Combarros O, Bullido MJ, Brouwers N, Bettens K, et al. (2010) The CALHM1 P86L polymorphism is a genetic modifier of age at onset in Alzheimer's disease: a meta-analysis study. *J Alzheimers Dis* **22:**247–255.

Langou K, Moumen A, Pellegrino C, Aebischer J, Medina I, Aebischer P, and Raoul C (2010) AAV-mediated expression of wild-type and ALS-linked mutant VAPB selectively triggers death of motoneurons through a $Ca^{2+}$-dependent ER-associated pathway. *J Neurochem* **114:**795–809.

Lara DR (2010) Caffeine, mental health, and psychiatric disorders. *J Alzheimers Dis* **20:**S239–S248.

Lashuel HA, Hartley D, Petre BM, Walz T, and Lansbury PT Jr (2002) Neurodegenerative disease: amyloid pores from pathogenic mutations. *Nature* **418:**291.

Lee KP, Yuan JP, Hong JH, So I, Worley PF, and Muallem S (2010) An endoplasmic reticulum/plasma membrane junction: STIM1/Orai1/TRPCs. *FEBS Lett* **584:**2022–2027.

Lehnart SE, Wehrens XH, and Marks AR (2005) Defective ryanodine receptor interdomain interactions may contribute to intracellular $Ca^{2+}$ leak: a novel therapeutic target in heart failure. *Circulation* **111:**3342–3346.

Leissring MA, Paul BA, Parker I, Cotman CW, and LaFerla FM (1999) Alzheimer's presenilin-1 mutation potentiates inositol 1,4,5-triphosphate-mediated calcium signaling in *Xenopus* oocytes. *J Neurochem* **72:**1061–1068.

Lencesova L, O'Neill A, Resneck WG, Bloch RJ, and Blaustein MP (2004) Plasma membrane-cytoskeleton-endoplasmic reticulum complexes in neurons and astrocytes. *J Biol Chem* **279:**2885–2893.

Lewis RS (2007) The molecular choreography of a store-operated calcium channel. *Nature* **446:**284–287.

Li W, Llopis J, Whitney M, Zlokarnik G, and Tsien RY (1998) Cell-permeant caged InsP3 ester shows that $Ca^{2+}$ spike frequency can optimize gene expression. *Nature* **392:**936–941.

Li X, Zima AV, Sheikh F, Blatter LA, and Chen J (2005) Endothelin-1-induced arrhythmogenic $Ca^{2+}$ signaling is abolished in atrial myocytes of inositol-1,4,5-trisphosphate(IP3)-receptor type 2-deficient mice. *Circ Res* **96:**1274–1281.

Liang G, Wang Q, Li Y, Kang B, Eckenhoff MF, Eckenhoff RG, and Wei H (2008) A presenilin-1 mutation renders neurons vulnerable to isoflurane toxicity. *Anesth Analg* **106:**492–500.

Lipskaia L, Chemaly ER, Hadri L, Lompre AM, and Hajjar RJ (2010) Sarcoplasmic reticulum $Ca^{2+}$ ATPase as a therapeutic target for heart failure. *Expert Opin Biol Ther* **10:**29–41.

Liu N, Colombi B, Memmi M, Zissimopoulos S, Rizzi N, Negri S, Imbriani M, Napolitano C, Lai FA, and Priori SG (2006) Arrhythmogenesis in catecholaminergic polymorphic ventricular tachycardia: insights from a RyR2 R4496C knock-in mouse model. *Circ Res* **99:**292–298.

Liu N and Priori SG (2008) Disruption of calcium homeostasis and arrhythmogenesis induced by mutations in the cardiac ryanodine receptor and calsequestrin. *Cardiovasc Res* **77:**293–301.

Liu YJ, Vieira E, and Gylfe E (2004) A store-operated mechanism determines the activity of the electrically excitable glucagon-secreting pancreatic alpha-cell. *Cell Calcium* **35:**357–365.

Lloyd-Evans E, Morgan AJ, He X, Smith DA, Elliot-Smith E, Sillence DJ, Churchill GC, Schuchman EH, Galione A, and Platt FM (2008) Niemann-Pick disease type C1 is a sphingosine storage disease that causes deregulation of lysosomal calcium. *Nat Med* **14:**1247–1255.

Luciani DS, Gwiazda KS, Yang TL, Kalynyak TB, Bychkivska Y, Frey MH, Jeffrey KD, Sampaio AV, Underhill TM, and Johnson JD (2009) Roles of IP3R and RyR $Ca^{2+}$ channels in endoplasmic reticulum stress and beta-cell death. *Diabetes* **58:**422–432.

Luik RM, Wang B, Prakriya M, Wu MM, and Lewis RS (2008) Oligomerization of STIM1 couples ER calcium depletion to CRAC channel activation. *Nature* **454:** 538–542.

Lytton J, Westlin M, Burk SE, Shull GE, and MacLennan DH (1992) Functional comparisons between isoforms of the sarcoplasmic or endoplasmic reticulum family of calcium pumps. *J Biol Chem* **267:**14483–14489.

MacDonald JF, Xiong ZG, and Jackson MF (2006) Paradox of $Ca^{2+}$ signaling, cell death and stroke. *Trends Neurosci* **29:**75–81.

Mackrill JJ (2010) Ryanodine receptor calcium channels and their partners as drug targets. *Biochem Pharmacol* **79:**1535–1543.

Maegawa GH, Stockley T, Tropak M, Banwell B, Blaser S, Kok F, Giugliani R, Mahuran D, and Clarke JT (2006) The natural history of juvenile or subacute GM2 gangliosidosis: 21 new cases and literature review of 134 previously reported. *Pediatrics* **118:**e1550–1562.

Maier LS, Zhang T, Chen L, DeSantiago J, Brown JH, and Bers DM (2003) Transgenic CaMKIIdeltaC overexpression uniquely alters cardiac myocyte $Ca^{2+}$ handling: reduced SR $Ca^{2+}$ load and activated SR $Ca^{2+}$ release. *Circ Res* **92:**904–911.

Mak DO and Foskett JK (1997) Single-channel kinetics, inactivation, and spatial distribution of inositol trisphosphate (IP3) receptors in Xenopus oocyte nucleus. *J Gen Physiol* **109:**571–587.

Mangoni ME and Nargeot J (2008) Genesis and regulation of the heart automaticity. *Physiol Rev* **88:**919–982.

Mank M, Santos AF, Direnberger S, Mrsic-Flogel TD, Hofer SB, Stein V, Hendel T, Reiff DF, Levelt C, Borst A, et al. (2008) A genetically encoded calcium indicator for chronic in vivo two-photon imaging. *Nat Methods* **5:**805–811.

Mark RJ, Lovell MA, Markesbery WR, Uchida K, and Mattson MP (1997a) A role for 4-hydroxynonenal, an aldehydic product of lipid peroxidation, in disruption of ion homeostasis and neuronal death induced by amyloid beta-peptide. *J Neurochem* **68:**255–264.

Mark RJ, Pang Z, Geddes JW, Uchida K, and Mattson MP (1997b) Amyloid beta-peptide impairs glucose transport in hippocampal and cortical neurons: involvement of membrane lipid peroxidation. *J Neurosci* **17:**1046–1054.

Mary V, Wahl F, Uzan A, and Stutzmann JM (2001) Enoxaparin in experimental stroke: neuroprotection and therapeutic window of opportunity. *Stroke* **32:**993–999.

Mattson MP (1990) Antigenic changes similar to those seen in neurofibrillary tangles are elicited by glutamate and $Ca^{2+}$ influx in cultured hippocampal neurons. *Neuron* **4:**105–117.

Mattson MP (1992) Calcium as sculptor and destroyer of neural circuitry. *Exp Gerontol* **27:**29–49.

Mattson MP (2003) Excitotoxic and excitoprotective mechanisms: abundant targets for the prevention and treatment of neurodegenerative disorders. *Neuromolecular Med* **3:**65–94.

Mattson MP (2004) Pathways towards and away from Alzheimer's disease. *Nature* **430:**631–639.

Mattson MP (2007) Calcium and neurodegeneration. *Aging Cell* **6:**337–350.

Mattson MP, Gleichmann M, and Cheng A (2008) Mitochondria in neuroplasticity and neurological disorders. *Neuron* **60:**748–766.

Mattson MP and Kroemer G (2003) Mitochondria in cell death: novel targets for neuroprotection and cardioprotection. *Trends Mol Med* **9:**196–205.

Mattson MP and Meffert MK (2006) Roles for NF-kappaB in nerve cell survival, plasticity, and disease. *Cell Death Differ* **13:**852–860.

Mattson MP, Rychlik B, Chu C, and Christakos S (1991) Evidence for calcium-reducing and excito-protective roles for the calcium-binding protein calbindin-D28k in cultured hippocampal neurons. *Neuron* **6:**41–51.

Mattson MP, Zhu H, Yu J, and Kindy MS (2000) Presenilin-1 mutation increases neuronal vulnerability to focal ischemia in vivo and to hypoxia and glucose deprivation in cell culture: involvement of perturbed calcium homeostasis. *J Neurosci* **20:**1358–1364.

Mellström B and Naranjo JR (2001) Mechanisms of Ca$^{2+}$-dependent transcription. *Curr Opin Neurobiol* 11:312–319.

Michalak M, Groenendyk J, Szabo E, Gold LI, and Opas M (2009) Calreticulin, a multi-process calcium-buffering chaperone of the endoplasmic reticulum. *Biochem J* **417:**651–666.

Micheletti R, Palazzo F, Barassi P, Giacalone G, Ferrandi M, Schiavone A, Moro B, Parodi O, Ferrari P, and Bianchi G (2007) Istaroxime, a stimulator of sarcoplasmic reticulum calcium adenosine triphosphatase isoform 2a activity, as a novel therapeutic approach to heart failure. *Am J Cardiol* **99:**24A–32A.

Mikoshiba K (2007) IP$_3$ receptor/Ca$^{2+}$ channel: from discovery to new signaling concepts. *J Neurochem* **102:**1426–1446.

Minster RL, Demirci FY, DeKosky ST, and Kamboh MI (2009) No association between CALHM1 variation and risk of Alzheimer disease. *Hum Mutat* **30:**E566–E569.

Miyakawa-Naito A, Uhlén P, Lal M, Aizman O, Mikoshiba K, Brismar H, Zelenin S, and Aperia A (2003) Cell signaling microdomain with Na,K-ATPase and inositol 1,4,5-trisphosphate receptor generates calcium oscillations. *J Biol Chem* **278:**50355–50361.

Mogami H, Tepikin AV, and Petersen OH (1998) Termination of cytosolic Ca$^{2+}$ signals: Ca$^{2+}$ reuptake into intracellular stores is regulated by the free Ca$^{2+}$ concentration in the store lumen. *EMBO J* **17:**435–442.

Muehlschlegel S and Sims JR (2009) Dantrolene: mechanisms of neuroprotection and possible clinical applications in the neurointensive care unit. *Neurocrit Care* **10:**103–115.

Müller WE, Koch S, Eckert A, Hartmann H, and Scheuer K (1995) beta-Amyloid peptide decreases membrane fluidity. *Brain Res* **674:**133–136.

Naidoo N (2009) ER and aging-protein folding and the ER stress response. *Ageing Res Rev* **8:**150–159.

Nakagawa M and Endo M (1984) Structures of xestospongin A, B, C and D, novel vasodilative compounds from marine sponge *Xestospongia exigua*. *Tetrahedron Lett* **25:**3227–3230.

Nakamura T, Barbara JG, Nakamura K, and Ross WN (1999) Synergistic release of Ca$^{2+}$ from IP3-sensitive stores evoked by synaptic activation of mGluRs paired with backpropagating action potentials. *Neuron* **24:**727–737.

Nakayama R, Yano T, Ushijima K, Abe E, and Terasaki H (2002) Effects of dantrolene on extracellular glutamate concentration and neuronal death in the rat hippocampal CA1 region subjected to transient ischemia. *Anesthesiology* **96:**705–710.

Nelson O, Supnet C, Liu H, and Bezprozvanny I (2010) Familial Alzheimer's disease mutations in presenilins: effects on endoplasmic reticulum calcium homeostasis and correlation with clinical phenotypes. *J Alzheimers Dis* **21:**781–793.

Nelson O, Tu H, Lei T, Bentahir M, de Strooper B, and Bezprozvanny I (2007) Familial Alzheimer disease-linked mutations specifically disrupt Ca$^{2+}$ leak function of presenilin 1. *J Clin Invest* **117:**1230–1239.

Nickson P, Toth A, and Erhardt P (2007) PUMA is critical for neonatal cardiomyocyte apoptosis induced by endoplasmic reticulum stress. *Cardiovasc Res* **73:**48–56.

Nicoud IB, Knox CD, Jones CM, Anderson CD, Pierce JM, Belous AE, Earl TM, and Chari RS (2007) 2-APB protects against liver ischemia-reperfusion injury by reducing cellular and mitochondrial calcium uptake. *Am J Physiol Gastrointest Liver Physiol* **293:**G623–G630.

Nishiyama M, Hong K, Mikoshiba K, Poo MM, and Kato K (2000) Calcium stores regulate the polarity and input specificity of synaptic modification. *Nature* **408:**584–588.

Norman E, Cutler RG, Flannery R, Wang Y, and Mattson MP (2010) Plasma membrane sphingomyelin hydrolysis increases hippocampal neuron excitability by sphingosine-1-phosphate mediated mechanisms. *J Neurochem* **114:**430–439.

Nunn DL and Taylor CW (1992) Luminal Ca$^{2+}$ increases the sensitivity of Ca$^{2+}$ stores to inositol 1,4,5-trisphosphate. *Mol Pharmacol* **41:**115–119.

Obenaus A, Mody I, and Baimbridge KG (1989) Dantrolene-Na (Dantrium) blocks induction of long-term potentiation in hippocampal slices. *Neurosci Lett* **98:**172–178.

Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R, Metherate R, Mattson MP, Akbari Y, and LaFerla FM (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular A$\beta$ and synaptic dysfunction. *Neuron* **39:**409–421.

Ohkubo N, Mitsuda N, Tamatani M, Yamaguchi A, Lee YD, Ogihara T, Vitek MP, and Tohyama M (2001) Apolipoprotein E4 stimulates cAMP response element-binding protein transcriptional activity through the extracellular signal-regulated kinase pathway. *J Biol Chem* **276:**3046–3053.

Oka T, Sato K, Hori M, Ozaki H, and Karaki H (2002) Xestospongin C, a novel blocker of IP3 receptor, attenuates the increase in cytosolic calcium level and degranulation that is induced by antigen in RBL-2H3 mast cells. *Br J Pharmacol* **135:**1959–1966.

Ong DS, Mu TW, Palmer AE, and Kelly JW (2010) Endoplasmic reticulum Ca$^{2+}$ increases enhance mutant glucocerebrosidase proteostasis. *Nat Chem Biol* **6:**424–432.

Orchard C and Brette F (2008) t-Tubules and sarcoplasmic reticulum function in cardiac ventricular myocytes. *Cardiovasc Res* **77:**237–244.

Pacifico F, Ulianich L, De Micheli S, Treglia S, Leonardi A, Vito P, Formisano S, Consiglio E, and Di Jeso B (2003) The expression of the sarco/endoplasmic reticulum Ca$^{2+}$-ATPases in thyroid and its down-regulation following neoplastic transformation. *J Mol Endocrinol* **30:**399–409.

Pani B, Ong HL, Brazer SC, Liu X, Rauser K, Singh BB, and Ambudkar IS (2009) Activation of TRPC1 by STIM1 in ER-PM microdomains involves release of the channel from its scaffold caveolin-1. *Proc Natl Acad Sci USA* **106:**20087–20092.

Pani B, Ong HL, Liu X, Rauser K, Ambudkar IS, and Singh BB (2008) Lipid rafts determine clustering of STIM1 in endoplasmic reticulum-plasma membrane junctions and regulation of store-operated Ca$^{2+}$ entry (SOCE). *J Biol Chem* **283:**17333–17340.

Paredes RM, Etzler JC, Watts LT, Zheng W, and Lechleiter JD (2008) Chemical calcium indicators. *Methods* **46:**143–151.

Park MK, Choi YM, Kang YK, and Petersen OH (2008) The endoplasmic reticulum as an integrator of multiple dendritic events. *Neuroscientist* **14:**68–77.

Parys JB, Missiaen L, De Smedt H, and Casteels R (1993) Loading dependence of inositol 1,4,5-trisphosphate-induced Ca$^{2+}$ release in the clonal cell line A7r5. Implications for the mechanism of quantal Ca$^{2+}$ release. *J Biol Chem* **268:**25206–25212.

Paschen W (2004) Endoplasmic reticulum dysfunction in brain pathology: critical role of protein synthesis. *Curr Neurovasc Res* **1:**173–181.

Pelled D, Lloyd-Evans E, Riebeling C, Jeyakumar M, Platt FM, and Futerman AH (2003) Inhibition of calcium uptake via the sarco/endoplasmic reticulum Ca$^{2+}$-ATPase in a mouse model of Sandhoff disease and prevention by treatment with *N*-butyldeoxynojirimycin. *J Biol Chem* **278:**29496–29501.

Petersen OH and Verkhratsky A (2007) Endoplasmic reticulum calcium tunnels integrate signalling in polarised cells. *Cell Calcium* **42:**373–378.

Pinton P, Giorgi C, Siviero R, Zecchini E, and Rizzuto R (2008) Calcium and apoptosis: ER-mitochondria Ca$^{2+}$ transfer in the control of apoptosis. *Oncogene* **27:**6407–6418.

Pollard HB, Rojas E, and Arispe N (1993) A new hypothesis for the mechanism of amyloid toxicity, based on the calcium channel activity of amyloid beta protein (A beta P) in phospholipid bilayer membranes. *Ann NY Acad Sci* **695:**165–168.

Prakriya M (2009) The molecular physiology of CRAC channels. *Immunol Rev* **231:**88–98.

Putney JW Jr (1987) Formation and actions of calcium-mobilizing messenger, inositol 1,4,5-trisphosphate. *Am J Physiol* **252:**G149–G157.

Qiu Z and Gruol DL (2003) Interleukin-6, beta-amyloid peptide and NMDA interactions in rat cortical neurons. *J Neuroimmunol* **139:**51–57.

Querfurth HW, Jiang J, Geiger JD, and Selkoe DJ (1997) Caffeine stimulates amyloid beta-peptide release from beta-amyloid precursor protein-transfected HEK293 cells. *J Neurochem* **69:**1580–1591.

Querfurth HW and Selkoe DJ (1994) Calcium ionophore increases amyloid beta peptide production by cultured cells. *Biochemistry* **33:**4550–4561.

Rácz G, Szabó A, Vér A, and Zádor E (2009) The slow sarco/endoplasmic reticulum Ca$^{2+}$ -ATPase declines independently of slow myosin in soleus muscle of diabetic rats. *Acta Biochim Pol* **56:**487–493.

Raymond CR and Redman SJ (2006) Spatial segregation of neuronal calcium signals encodes different forms of LTP in rat hippocampus. *J Physiol* **570:**97–111.

Renner M, Lacor PN, Velasco PT, Xu J, Contractor A, Klein WL, and Triller A (2010) Deleterious effects of amyloid beta oligomers acting as an extracellular scaffold for mGluR5. *Neuron* **66:**739–754.

Renvoisé B and Blackstone C (2010) Emerging themes of ER organization in the development and maintenance of axons. *Curr Opin Neurobiol* **20:**531–537.

Rich MW (2006) Heart failure in older adults. *Med Clin North Am* **90:**863–885.

Rochefort NL and Konnerth A (2008) Genetically encoded Ca$^{2+}$ sensors come of age. *Nature Methods* **5:**761–762.

Roderick HL, Lechleiter JD, and Camacho P (2000) Cytosolic phosphorylation of calnexin controls intracellular Ca$^{2+}$ oscillations via an interaction with SERCA2b. *J Cell Biol* **149:**1235–1248.

Rodriguez D, Rojas-Rivera D, and Hetz C (2011) Integrating stress signals at the endoplasmic reticulum: the BCL-2 protein family rheostat. *Biochim Biophys Acta* 1813:564–574.

Rome LC (2006) Design and function of superfast muscles: new insights into the physiology of skeletal muscle. *Annu Rev Physiol* **68:**193–221.

Rosenberg H, Davis M, James D, Pollock N, and Stowell K (2007) Malignant hyperthermia. *Orphanet J Rare Dis* **2:**21.

Ross WN, Nakamura T, Watanabe S, Larkum M, and Lasser-Ross N (2005) Synaptically activated Ca$^{2+}$ release from internal stores in CNS neurons. *Cell Mol Neurobiol* **25:**283–295.

Rothstein JD (2009) Current hypotheses for the underlying biology of amyotrophic lateral sclerosis. *Ann Neurol* **65:**S3–S9.

Rybalchenko V, Hwang SY, Rybalchenko N, and Koulen P (2008) The cytosolic N-terminus of presenilin-1 potentiates mouse ryanodine receptor single channel activity. *Int J Biochem Cell Biol* **40:**84–97.

Schapansky J, Olson K, Van Der Ploeg R, and Glazner G (2007) NF-kappaB activated by ER calcium release inhibits Abeta-mediated expression of CHOP protein: enhancement by AD-linked mutant presenilin 1. *Exp Neurol* **208:**169–176.

Scheff SW, Price DA, Schmitt FA, and Mufson EJ (2006) Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. *Neurobiol Aging* **27:**1372–1384.

Schoenenberger P, Schärer YP, and Oertner TG (2011) Channelrhodopsin as a tool to study synaptic transmission and plasticity. *Exp Physiol* **96:**34–39.

Selkoe DJ (2002) Alzheimer's disease is a synaptic failure. *Science* **298:**789–791.

Sharp AH, McPherson PS, Dawson TM, Aoki C, Campbell KP, and Snyder SH (1993) Differential immunohistochemical localization of inositol-1,4,5-trisphosphate- ad ryanodine-sensitive $Ca^{2+}$ release in channels in rat brain. *J Neurosci* **13:**3051–3063.

Sheehan JP, Swerdlow RH, Parker WD, Miller SW, Davis RE, and Tuttle JB (1997) Altered calcium homeostasis in cells transformed by mitochondria from individuals with Parkinson's disease. *J Neurochem* **68:**1221–1233.

Shibata Y, Voeltz GK, and Rapoport TA (2006) Rough sheets and smooth tubules. *Cell* **126:**435–439.

Shmigol A, Svichar N, Kostyuk P, and Verkhratsky A (1996) Gradual caffeine-induced $Ca^{2+}$ release in mouse dorsal root ganglion neurons is controlled by cytoplasmic and luminal $Ca^{2+}$. *Neuroscience* **73:**1061–1067.

Shtifman A, Ward CW, Laver DR, Bannister ML, Lopez JR, Kitazawa M, LaFerla FM, Ikemoto N, and Querfurth HW (2010) Amyloid-$\beta$ protein impairs $Ca^{2+}$ release and contractility in skeletal muscle. *Neurobiol Aging* **31:**2080–2090.

Sirabella R, Secondo A, Pannaccione A, Scorziello A, Valsecchi V, Adornetto A, Bilo L, Di Renzo G, and Annunziato L (2009) Anoxia-induced NF-kappaB-dependent upregulation of NCX1 contributes to $Ca^{2+}$ refilling into endoplasmic reticulum in cortical neurons. *Stroke* **40:**922–929.

Sitsapesan R and Williams AJ (1997) Regulation of current flow through ryanodine receptors by luminal $Ca^{2+}$. *J Membr Biol* **159:**179–185.

Smith FL, Lohmann AB, and Dewey WL (1999) Involvement of phospholipid signal transduction pathways in morphine tolerance in mice. *Br J Pharmacol* **128:**220–226.

Smith IF, Hitt B, Green KN, Oddo S, and LaFerla FM (2005) Enhanced caffeine-induced $Ca^{2+}$ release in the 3xTg-AD mouse model of Alzheimer's disease. *J Neurochem* **94:**1711–1718.

Smyth JT, Hwang SY, Tomita T, DeHaven WI, Mercer JC, and Putney JW (2010) Activation and regulation of store-operated calcium entry. *J Cell Mol Med* **14:**2337–2349.

Smyth JT, Petranka JG, Boyles RR, DeHaven WI, Fukushima M, Johnson KL, Williams JG, and Putney JW Jr (2009) Phosphorylation of STIM1 underlies suppression of store-operated calcium entry during mitosis. *Nat Cell Biol* **11:**1465–1472.

Snetkov VA, Aaronson PI, Ward JP, Knock GA, and Robertson TP (2003) Capacitative calcium entry as a pulmonary specific vasoconstrictor mechanism in small muscular arteries of the rat. *Br J Pharmacol* **140:**97–106.

Solovyova N, Fernyhough P, Glazner G, and Verkhratsky A (2002a) Xestospongin C empties the ER calcium store but does not inhibit InsP3-induced $Ca^{2+}$ release in cultured dorsal root ganglia neurones. *Cell Calcium* **32:**49–52.

Solovyova N and Verkhratsky A (2002) Monitoring of free $Ca^{2+}$ in the neuronal endoplasmic reticulum: an overview of modern approaches. *J Neurosci Methods* **122:**1–12.

Solovyova N, Veselovsky N, Toescu EC, and Verkhratsky A (2002b) $Ca^{2+}$ dynamics in the lumen of the endoplasmic reticulum in sensory neurons: direct visualization of $Ca^{2+}$-induced $Ca^{2+}$ release triggered by physiological $Ca^{2+}$ entry. *EMBO J* **21:**622–630.

Stevens FJ and Argon Y (1999) Protein folding in the ER. *Semin Cell Dev Biol* **10:**443–454.

Stutzmann GE (2005) Calcium dysregulation, $IP_3$ signaling, and Alzheimer's disease. *The Neuroscientist* **11:**110–115.

Stutzmann GE (2007) The pathogenesis of Alzheimer's disease–is it a lifelong "calciumopathy"? *The Neuroscientist* **13:**546–559.

Stutzmann GE, LaFerla FM, and Parker I (2003) $Ca^{2+}$ signaling in mouse cortical neurons studied by two-photon imaging and photoreleased inositol triphosphate. *J Neurosci* **23:**758–765.

Stutzmann GE, Smith I, Caccamo A, Oddo S, Laferla FM, and Parker I (2006) Enhanced ryanodine receptor recruitment contributes to $Ca^{2+}$ disruptions in young, adult, and aged Alzheimer's disease mice. *J Neurosci* **26:**5180–5189.

Sumbilla C, Cavagna M, Zhong L, Ma H, Lewis D, Farrance I, and Inesi G (1999) Comparison of SERCA1 and SERCA2a expressed in COS-1 cells and cardiac myocytes. *Am J Physiol* **277:**H2381–H2391.

Supnet C, Grant J, Kong H, Westaway D, and Mayne M (2006) Amyloid-beta-$_{(1-42)}$ increases ryanodine receptor-3 expression and function in neurons of TgCRND8 mice. *J Biol Chem* **281:**38440–38447.

Supnet C, Noonan C, Richard K, Bradley J, and Mayne M (2010) Up-regulation of the type 3 ryanodine receptor is neuroprotective in the TgCRND8 mouse model of Alzheimer's disease. *J Neurochem* **112:**356–365.

Talukder MA, Kalyanasundaram A, Zuo L, Velayutham M, Nishijima Y, Periasamy M, and Zweier JL (2008) Is reduced SERCA2a expression detrimental or beneficial to postischemic cardiac function and injury? Evidence from heterozygous SERCA2a knockout mice. *Am J Physiol Heart Circ Physiol* **294:**H1426–H1434.

Tang TS, Slow E, Lupu V, Stavrovskaya IG, Sugimori M, Llinás R, Kristal BS, Hayden MR, and Bezprozvanny I (2005) Disturbed $Ca^{2+}$ signaling and apoptosis of medium spiny neurons in Huntington's disease. *Proc Natl Acad Sci* **102:**2602–2607.

Tang Y, Li H, and Liu JP (2010) Niemann-Pick disease type C: from molecule to clinic. *Clin Exp Pharmacol Physiol* **37:**132–140.

Tavakoli M and Malik RA (2008) Management of painful diabetic neuropathy. *Expert Opin Pharmacother* **9:**2969–2978.

Terasaki M, Slater NT, Fein A, Schmidek A, and Reese TS (1994) Continuous network of endoplasmic reticulum in cerebellar Purkinje neurons. *Proc Natl Acad Sci USA* **91:**7510–7751.

Terentyev D, Nori A, Santoro M, Viatchenko-Karpinski S, Kubalova Z, Gyorke I, Terentyeva R, Vedamoorthyrao S, Blom NA, Valle G, et al. (2006) Abnormal interactions of calsequestrin with the ryanodine receptor calcium release channel complex linked to exercise-induced sudden cardiac death. *Circ Res* **98:**1151–1158.

Tester DJ, Dura M, Carturan E, Reiken S, Wronska A, Marks AR, and Ackerman MJ (2007) A mechanism for sudden infant death syndrome (SIDS): stress-induced leak via ryanodine receptors. *Heart Rhythm* **4:**733–739.

Thomas NL, Maxwell C, Mukherjee S, and Williams AJ (2010) Ryanodine receptor mutations in arrhythmia: the continuing mystery of channel dysfunction. *FEBS Lett* **584:**2153–2160.

Thomas-Virnig CL, Sims PA, Simske JS, and Hardin J (2004) The inositol 1,4,5-trisphosphate receptor regulates epidermal cell migration in *Caenorhabditis elegans*. *Curr Biol* **14:**1882–1887.

Thrower EC, Hagar RE, and Ehrlich BE (2001) Regulation of Ins(1,4,5)P3 receptor isoforms by endogenous modulators. *Trends Pharmacol Sci* **22:**580–586.

Tian L, Hires SA, Mao T, Huber D, Chiappe ME, Chalasani SH, Petreanu L, Akerboom J, McKinney SA, Schreiter ER, et al. (2009) Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. *Nat Methods* **6:**875–881.

Toescu EC, O'Neill SC, Petersen OH, and Eisner DA (1992) Caffeine inhibits the agonist-evoked cytosolic $Ca^{2+}$ signal in mouse pancreatic acinar cells by blocking inositol trisphosphate production. *J Biol Chem* **267:**23467–23470.

Tolar M, Keller JN, Chan S, Mattson MP, Marques MA, and Crutcher KA (1999) Truncated apolipoprotein E (ApoE) causes increased intracellular calcium and may mediate ApoE neurotoxicity. *J Neurosci* **19:**7100–7110.

Tong J, Oyamada H, Demaurex N, Grinstein S, McCarthy TV, and MacLennan DH (1997) Caffeine and halothane sensitivity of intracellular $Ca^{2+}$ release is altered by 15 calcium release channel (ryanodine receptor) mutations associated with malignant hyperthermia and/or central core disease. *J Biol Chem* **272:**26332–26339.

Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, Hansen KB, Yuan H, Myers SJ, and Dingledine R (2010) Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol Rev* **62:**405–496.

Treves S, Anderson AA, Ducreux S, Divet A, Bleunven C, Grasso C, Paesante S, and Zorzato F (2005) Ryanodine receptor 1 mutations, dysregulation of calcium homeostasis and neuromuscular disorders. *Neuromuscul Disord* **15:**577–587.

Tu H, Nelson O, Bezprozvanny A, Wang Z, Lee SF, Hao YH, Serneels L, De Strooper B, Yu G, and Bezprozvanny I (2006) Presenilins form ER $Ca^{2+}$ leak channels, a function disrupted by familial Alzheimer's disease-linked mutations. *Cell* **126:**981–993.

Tu H, Wang Z, and Bezprozvanny I (2005) Modulation of mammalian inositol 1,4,5-trisphosphate receptor isoforms by calcium: a role of calcium sensor region. *Biophys J* **88:**1056–1069.

Uchida K, Aramaki M, Nakazawa M, Yamagishi C, Makino S, Fukuda K, Nakamura T, Takahashi T, Mikoshiba K, and Yamagishi H (2010) Gene knock-outs of inositol 1,4,5-trisphosphate receptors types 1 and 2 result in perturbation of cardiogenesis. *PLoS One* **5:**e12500.

Uchiyama K and Kondo H (2005) p97/p47-mediated biogenesis of Golgi and ER. *J Biochem* **137:**115–119.

Vanden Abeele F, Bidaux G, Gordienko D, Beck B, Panchin YV, Baranova AV, Ivanov DV, Skryma R, and Prevarskaya N (2006) Functional implications of calcium permeability of the channel formed by pannexin 1. *J Cell Biol* **174:**535–546.

Verdurand M, Bérod A, Le Bars D, and Zimmer L (2011) Effects of amyloid-$\beta$ peptides on the serotoninergic 5-HT1A receptors in the rat hippocampus. *Neurobiol Aging* **32:**103–114.

Verkhratsky A (2002) The endoplasmic reticulum and neuronal calcium signaling. *Cell Calcium* **32:**393–404.

Verkhratsky A (2005) Physiology and pathophysiology of calcium store in the endoplasmic reticulum of neurons. *Physiol Rev* **85:**201–279.

Verkhratsky A and Toescu EC (2003) Endoplasmic reticulum $Ca^{2+}$ homeostasis and neuronal death. *J Cell Mol Med* **7:**351–361.

Viatchenko-Karpinski S, Terentyev D, Györke I, Terentyeva R, Volpe P, Priori SG, Napolitano C, Nori A, Williams SC, and Györke S (2004) Abnormal calcium signaling and sudden cardiac death associated with mutation of calsequestrin. *Circ Res* **94:**471–477.

Vinogradova TM, Brochet DX, Sirenko S, Li Y, Spurgeon H, and Lakatta EG (2010) Sarcoplasmic reticulum $Ca^{2+}$ pumping kinetics regulates timing of local $Ca^{2+}$ releases and spontaneous beating rate of rabbit sinoatrial node pacemaker cells. *Circ Res* **107:**767–775.

Vitner EB, Platt FM, and Futerman AH (2010) Common and uncommon pathogenic cascades in lysosomal storage diseases. *J Biol Chem* **285:**20423–20427.

Voeltz GK, Prinz WA, Shibata Y, Rist JM, and Rapoport TA (2006) A class of membrane proteins shaping the tubular endoplasmic reticulum. *Cell* **124:**573–586.

Wang F, Agnello G, Sotolongo N, and Segatori L (2011) $Ca^{2+}$ homeostasis modulation enhances the amenability of L444P glucosylcerebrosidase to proteostasis regulation in patient-derived fibroblasts. *ACS Chem Biol* **6:**158–168.

Wang Y, Greig NH, Yu QS, and Mattson MP (2009) Presenilin-1 mutation impairs cholinergic modulation of synaptic plasticity and suppresses NMDA currents in hippocampus slices. *Neurobiol Aging* **30:**1061–1068.

Watanabe S, Hong M, Lasser-Ross N, and Ross WN (2006) Modulation of calcium wave propagation in the dendrites and to the soma of rat hippocampal pyramidal neurons. *J Physiol* **575:**455–468.

Wehrens XH, Lehnart SE, Huang F, Vest JA, Reiken SR, Mohler PJ, Sun J, Guatimosim S, Song LS, Rosemblit N, et al. (2003) FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death. *Cell* **113:**829–840.

Wei H and Perry DC (1996) Dantrolene is cytoprotective in two models of neuronal cell death. *J Neurochem* **67:**2390–2398.

Wheeler D, Knapp E, Bandaru VV, Wang Y, Knorr D, Poirier C, Mattson MP, Geiger JD, and Haughey NJ (2009) Tumor necrosis factor-alpha-induced neutral sphingomyelinase-2 modulates synaptic plasticity by controlling the membrane insertion of NMDA receptors. *J Neurochem* **109:**1237–1249.

Wilms CD and Häusser M (2009) Lighting up neural networks using a new generation of genetically encoded calcium sensors. *Nature Methods* **6:**871–872.

Won JG and Orth DN (1995) Role of inositol trisphosphate-sensitive calcium stores in the regulation of adrenocorticotropin secretion by perifused rat anterior pituitary cells. *Endocrinology* **136:**5399–5408.

Wu J, Holstein JD, Upadhyay G, Lin DT, Conway S, Muller E, and Lechleiter JD (2007) Purinergic receptor-stimulated IP3-mediated $Ca^{2+}$ release enhances neuroprotection by increasing astrocyte mitochondrial metabolism during aging. *J Neurosci* **27:**6510–6520.

Wu L, Katz S, and Brown GR (1994) Inositol 1,4,5-trisphosphate-, GTP-, arachidonic acid- and thapsigargin-mediated intracellular calcium movement in PANC-1 microsomes. *Cell Calcium* **15:**228–240.

Xing H, Azimi-Zonooz A, Shuttleworth CW, and Connor JA (2004) Caffeine releasable stores of $Ca^{2+}$ show depletion prior to the final steps in delayed CA1 neuronal death. *J Neurophysiol* **92:**2960–2967.

Yamashita M, Oki Y, Iino K, Hayashi C, Yogo K, Matsushita F, Sasaki S, and Nakamura H (2009) The role of store-operated $Ca^{2+}$ channels in adrenocorticotropin release by rat pituitary cells. *Regul Pept* **156:**57–64.

Yang HT, Tweedie D, Wang S, Guia A, Vinogradova T, Bogdanov K, Allen PD, Stern MD, Lakatta EG, and Boheler KR (2002) The ryanodine receptor modulates the spontaneous beating rate of cardiomyocytes during development. *Proc Natl Acad Sci USA* **99:**9225–9230.

Yano M, Ono K, Ohkusa T, Suetsugu M, Kohno M, Hisaoka T, Kobayashi S, Hisamatsu Y, Yamamoto T, Kohno M, et al. (2000) Altered stoichiometry of FKBP12.6 versus ryanodine receptor as a cause of abnormal $Ca^{2+}$ leak through ryanodine receptor in heart failure. *Circulation* **102:**2131–2136.

Yasuda R, Sabatini BL, and Svoboda K (2003) Plasticity of calcium channels in dendritic spines. *Nat Neurosci* **6:**948–955.

Young RR (1987) Physiologic and pharmacologic approaches to spasticity. *Neurol Clin* **5:**529–539.

Yu Z, Luo H, Fu W, and Mattson MP (1999) The endoplasmic reticulum stress-responsive protein GRP78 protects neurons against excitotoxicity and apoptosis: suppression of oxidative stress and stabilization of calcium homeostasis. *Exp Neurol* **155:**302–314.

Yule DI, Betzenhauser MJ, and Joseph SK (2010) Linking structure to function: recent lessons from inositol 1,4,5-trisphosphate receptor mutagenesis. *Cell Calcium* **47:**469–479.

Yuste R, Majewska A, and Holthoff K (2000) From form to function: calcium compartmentalization in dendritic spines. *Nat Neurosci* **3:**653–659.

Zai L, Ferrari C, Subbaiah S, Havton LA, Coppola G, Strittmatter S, Irwin N, Geschwind D, and Benowitz LI (2009) Inosine alters gene expression and axonal projections in neurons contralateral to a cortical infarct and improves skilled use of the impaired limb. *J Neurosci* **29:**8187–8197.

Zempel H, Thies E, Mandelkow E, and Mandelkow EM (2010) Abeta oligomers cause localized $Ca^{2+}$ elevation, missorting of endogenous Tau into dendrites, Tau phosphorylation, and destruction of microtubules and spines. *J Neurosci* **30:**11938–11950.

Zeng Y, Lv XH, Zeng SQ, Tian SL, Li M, and Shi J (2008) Sustained depolarization-induced propagation of $[Ca^{2+}]$i oscillations in cultured DRG neurons: the involvement of extracellular ATP and P2Y receptor activation. *Brain Res* **1239:**12–23.

Zhang H, Sun S, Herreman A, De Strooper B, and Bezprozvanny I (2010) Role of presenilins in neuronal calcium homeostasis. *J Neurosci* **30:**8566–8580.

Zucker RS and Regehr WG (2002) Short-term synaptic plasticity. *Annu Rev Physiol* **64:**355–405.