J. Cell. Mol. Med. Vol 7, No 4, 2003 pp. 351-361

Neuroscience Review Series

Endoplasmic reticulum Ca^{2+} homeostasis and neuronal death

A. Verkhratsky [a], E. C. Toescu [b]

[a] The University of Manchester, School of Biological Sciences, Manchester, United Kingdom
[b] University of Birmingham, School of Medicine, Birmingham, United Kingdom

Received: October 29, 2003; Accepted: November 27, 2003

Abstract

The endoplasmic reticulum (ER) is a universal signalling organelle, which regulates a wide range of neuronal functional responses. Calcium release from the ER underlies various forms of intracellular Ca^{2+} signalling by either amplifying Ca^{2+} entry through voltage-gated Ca^{2+} channels by Ca^{2+}-induced Ca^{2+} release (CICR) or by producing local or global cytosolic calcium fluctuations following stimulation of metabotropic receptors through inositol-1,4,5-trisphosphate-induced Ca^{2+} release (IICR). The ER Ca^{2+} store emerges as a single interconnected pool, thus allowing for a long-range Ca^{2+} signalling via intra-ER tunnels. The fluctuations of intra-ER free Ca^{2+} concentration regulate the activity of numerous ER resident proteins responsible for post-translational protein folding and modification. Disruption of ER Ca^{2+} homeostasis results in the developing of ER stress response, which in turn controls neuronal survival. Altered ER Ca^{2+} handling may be involved in pathogenesis of various neurodegenerative diseases including brain ischemia and Alzheimer dementia.

Keywords: Ca^{2+} - Ca^{2+} release - Endoplasmic reticulum - InsP3 - ryanodine - neurodegeneration

* Correspondence to: Prof. A. VERKHRATSKY,
The University of Manchester, School of Biological Sciences,
1.124 Stopford Building, Oxford Road,
Manchester M13 9PT, United Kingdom.
Tel.: (+44 161) 2755414, Fax: (+44 161) 275 5948.
Email: alex.verkhratsky@man.ac.uk

Introduction

Fluctuations of free Ca^{2+} concentration in various cellular subcompartments appear as a universal signalling system, which by employing a wide array of Ca^{2+}-dependent effectors, regulate most of the cellular functions (see e.g. [1-7]). Indeed, almost every cellular response, from contraction and exocytosis to gene expression and cell death is controlled by localised or global changes in free Ca^{2+} concentration within the cytosol, as well as Ca^{2+} tides and ebbs within various organelles, such as mitochondria, nucleus and endoplasmic reticulum. For many years the cytosolic Ca^{2+} signals received a particular attention, so that local Ca^{2+} domains became accountable for exocytosis and regulation of plasmalemmal channels, whereas global propagating Ca^{2+} waves were identified as means for inter- and intracellular communication. Very recently however, dynamic Ca^{2+} changes within the specific intracellular compartment, the endoplasmic reticulum (ER) attracted much attention, as these changes became implicated in regulation of many cellular functions, from rapid signalling to long-lasting adaptive responses, and determining the cell fate. In this review we will outline the importance of the ER as a universal signalling organelle, and summarise the role of ER Ca^{2+} homeostasis in neuronal pathology.

ER as a universal signalling organelle

The endoplasmic reticulum (ER) is an intracellular organelle of fundamental importance, represented by an extensive network formed by cisternae and microtubules, which stretches from the nuclear envelope to the cell surface in all eukaryotic cells [8, 9]. The endoplasmic reticulum is most likely the largest organelle, with endomembrane accounting for more than 50% of all the cellular membranes, and occupies a substantial part (> 10 %) of the cell volume. The ER plays several vital functions. Firstly, the ER is the place for protein synthesis in the rough endoplasmic reticulum and correct post-translational "folding" of these proteins [10]. Secondly, the ER serves as a common transport route by which numerous proteins are delivered to their destination [11]. Thirdly, the ER acts as an indispensable source for fast physiological signalling being a dynamic Ca^{2+} reservoir, which can be activated by both electrical and chemical cell stimulation [2, 12-15]. Moreover, it emerges that the ER plays a key role in redistributing Ca^{2+} ions within the cell, as the excitable ER membrane forms propagating cytosolic Ca^{2+} waves [16] and the ER lumen functions as an intracellular Ca^{2+} tunnel [17].

All these processes demonstrate a high degree of co-ordination and therefore the ER can be regarded as a signalling organelle capable of receiving, integrating and transmitting various signals [2, 14]. The input signals to the ER are many (Fig. 1) and they include Ca^{2+} ions, cytoplasmic messengers such as cADPR and $InsP_3$, reactive oxygen species, and various proteins synthesised within the ER lumen [14]. The output signals are represented by Ca^{2+} ions released from the ER via intracellular Ca^{2+} channels, various transcription factors (e.g. NF-κB) and factors of stress response [18, 19]. The nature of the integrative mechanisms taking place within the ER lumen is yet to be fully characterised, however a substantial part of intra-ER integration is likely to be regulated by Ca^{2+} ions. Indeed, intraluminal space contains a high concentration of free Ca^{2+}, varying from 0.2–1 mM [20-22]. Simultaneously, high intraluminal free Ca^{2+} concentration appears to be a key factor determining the activity of synthesis and processing of proteins within the endoplasmic reticulum [23]. Within the framework of this theory, the Ca^{2+} ion emerges as a messenger molecule which integrates various signals within the endoplasmic reticulum: the fluctuations of $[Ca^{2+}]_L$ induced by signals originating at the level of the plasmalemma (i.e. Ca^{2+} entry or activation of metabotropic receptors) regulate in turn protein synthesis and processing via generating secondary signalling events between the endoplasmic reticulum and the nucleus. Disruption of endoplasmic reticulum Ca^{2+} homeostasis triggers endoplasmic reticulum stress response [24]. When deficits of endoplasmic reticulum Ca^{2+} handling are severe and persisting the endoplasmic reticulum becomes a source of cell death signals. This is particularly important for numerous pathological processes, and disruption of ER Ca^{2+} homeostasis may be implicated as a trigger factor in many forms of cellular pathology [25-27].

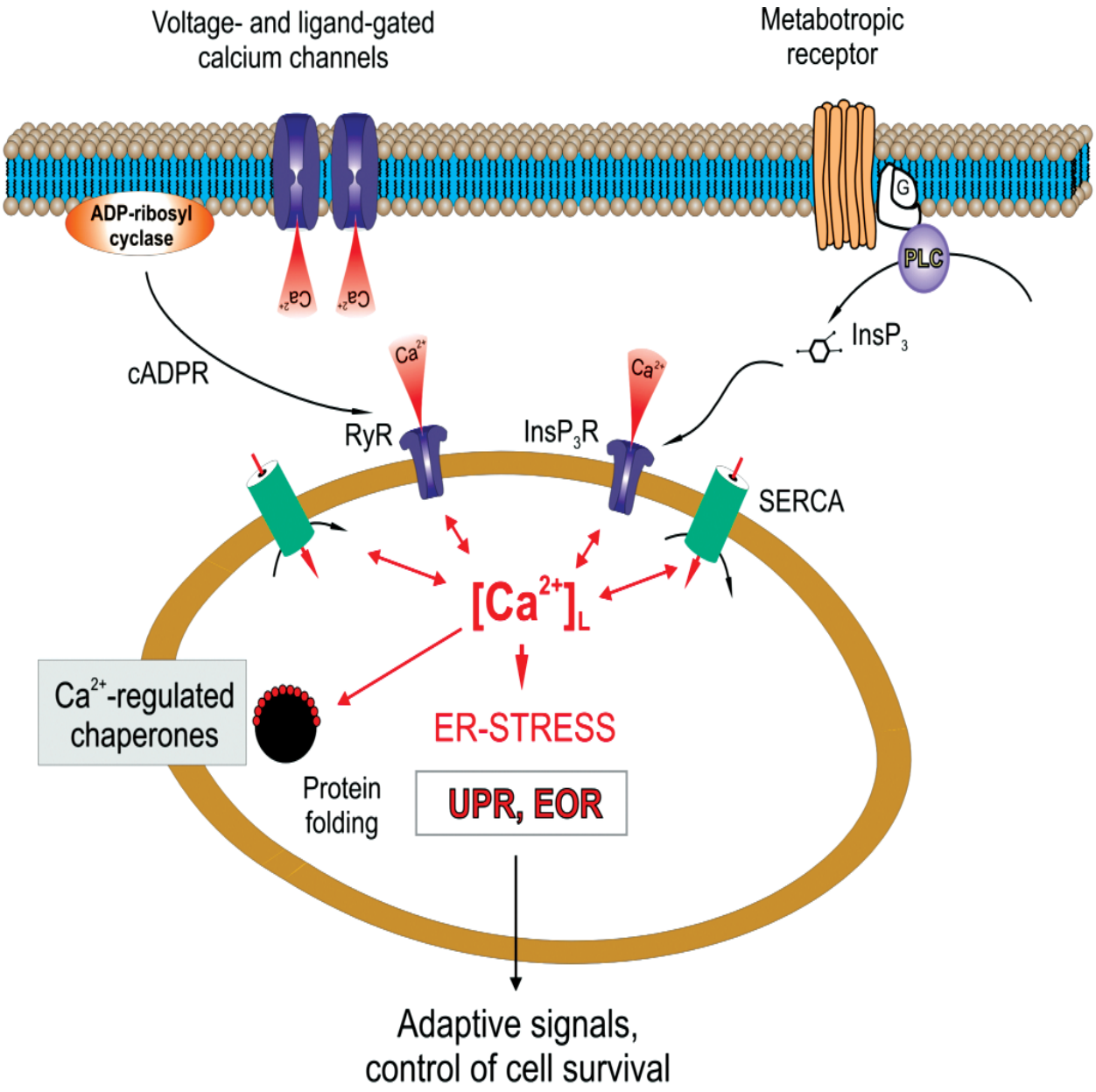

Fig. 1 Signal integration within the ER: intraluminal free Ca^{2+} assumes the leading role. The intraluminal free Ca^{2+} concentration is determined by the balance between Ca^{2+} transport across the endomembrane and intra-ER Ca^{2+} buffering. Ca^{2+} transport is the function of ER Ca^{2+} channels (the $InsP_3$ receptor, $InsP_3R$ and the ryanodine receptor, RyR) and ER Ca^{2+} pump of SERCA (Sarco(Endo)Plasmic reticulum ATPase) type. Functional activity of $InsP_3Rs$ is controlled by both $InsP_3$ and cytosolic free Ca^{2+}, whereas RyRs are directly activated by $[Ca^{2+}]_i$ and may be modulated by cyclic ADP rybose (cADPR). Intraluminal concentration of free Ca^{2+} regulates the availability of Ca^{2+} release channels (rise in $[Ca^{2+}]_L$ increases the sensitivity of RyRs/$InsP_3Rs$ to $[Ca^{2+}]_i$ and/or $InsP_3$) and the activity of SERCA (decrease in $[Ca^{2+}]_L$ dramatically increases the velocity of SERCA-dependent Ca^{2+} pumping across the endomembrane). Disruption of ER Ca^{2+} homeostasis affects $[Ca^{2+}]_L$, which in turn alters functional activity of Ca^{2+}-dependent chaperones responsible for post-translational protein processing and activates ER stress response; the latter being manifested in two forms, the unfolded protein response (UPR) and endoplasmic reticulum overload response (EOR). The ER stress activates numerous signals accountable for long-lasting adaptive responses, including cell survival/death signals.

The ER and neuronal Ca^{2+} signalling

The function of ER as a Ca^{2+} signalling organelle is executed by several families of proteins localised in the endomembrane as well as within the ER lumen, specifically by Ca^{2+} pumps of SERCA (Sarco (Endo) PlasmicReticulum calcium ATPase) type [28], Ca^{2+} release channels and intraluminal Ca^{2+} binding proteins, many of which also serve as Ca^{2+}-regulated enzymes (see e.g. [15, 29]). Out of the several types of currently known Ca^{2+} release channels (ryanodine receptors - RyRs, $InsP_3$ receptors - $InsP_3Rs$; NAADP receptors - NAADPRs, see [30-33] for review) all three types of RyRs and all three types of $InsP_3Rs$ are found in the central and peripheral nervous system [20]. The NAADP receptors were found in brain microsomes [34] and NAADP-regulated Ca^{2+} release was described in neurones from buccal ganglion of *Aplysia californica* [35], yet their relevance for Ca^{2+} signalling in vertebrate neurones is still not yet established.

Initial studies of the role of ER Ca^{2+} stores in neuronal signalling employed electrophysiological recordings from single cultured neurones. These experiments found that treatment of sympathetic neurones with caffeine, a known activator of ryanodine receptors, led to the activation of Ca^{2+}-dependent K^+ conductance, manifested in rhythmic hyperpolarisations. Activation of Ca^{2+}-dependent K^+ channels was inhibited by dantrolene, thus linking this process to Ca^{2+} release from the ER [36, 37]. Later on, the similar activation of Ca^{2+}-dependent K^+ channels resulted from a caffeine-induced release of intracellular Ca^{2+} was also described in several other types of neurones from both invertebrates and vertebrates (e.g. [36, 38, 39]). Introduction of Ca^{2+}-sensitive fluorescent probes [40] allowed Neering and McBurney to demonstrate, for the first time, the cytosolic Ca^{2+} increase following application of caffeine to single mammalian peripheral neurones. This $[Ca^{2+}]_i$ elevation did not require extracellular Ca^{2+}, being thus solely produced by a release of Ca^{2+} from intracellular store [41]. This key observation was subsequently confirmed in numerous experiments on acutely dissociated or cultured neurones from invertebrates and mammals, isolated from peripheral [42-48], as well as from the central nervous system [49, 50]. All these studies have convincingly demonstrated that caffeine in 10 - 20 mM concentration applied in a Ca^{2+}-free extracellular solution, triggers transient increase in $[Ca^{2+}]_i$, which is sensitive to pharmacological agents known to interact with the ER Ca^{2+} movements (e.g. ryanodine, dantrolene, thapsigargin and ruthenium red - see [51-54]). Furthermore, the caffeine-induced Ca^{2+} release from the ER was identified in experiments *in situ* in brain slices [55-57] substantiating thus the physiological relevance of neuronal ER Ca^{2+} store.

Simultaneously the $InsP_3$-induced Ca^{2+} release (IICR) in neurones was also identified. Initial findings have shown that stimulation of metabotropic receptors in sympathetic neurones by acetylcholine, substance P and LHRH increased intracellular $InsP_3$ levels and initiated intracellular Ca^{2+} release [58]. Morphological studies localised the $InsP_3$ receptors in many different neurones and showed their appearance in neuronal sub-compartments, including somata, axons and dendrites [59, 60]. Further experiments demonstrated that the UV photolysis of exogenous "caged" $InsP_3$, dialysed into a single Purkinje neurone via patch pipette, resulted in Ca^{2+} release from the ER [61, 62]. Later on, the IICR triggered by stimulation of various metabotropic receptors was described in several types of isolated peripheral and central neurones [63, 64], as well as in neurones in acute brain slices (see e.g. [55, 65]).

Ca^{2+} release and intra-ER Ca^{2+} dynamics

The level and dynamic changes in the free Ca^{2+} concentration within the ER lumen ($[Ca^{2+}]_L$) determines the ER function as a Ca^{2+} signalling organelle, and regulates the activity of intra-ER resident enzymatic cascades. A number of experimental techniques for monitoring $[Ca^{2+}]_L$ level were developed recently. Two distinct technical approaches were proposed so far. The first method employed cell transfection with an endoplasmic reticulum-targeted Ca^{2+} probe, such as the luminescent indicator aequorin [66] or fluorescent indicators based on fluorescence resonance energy transfer between two modified fluorescent proteins (e.g. green and blue) known as "cameleon" probes [67]. The alternative approach used "conventional" low-affinity Ca^{2+}-sensitive fluorescent probes such as Mag-Fura-2, Mag-Fluo-4, Fluo-3FF etc. with K_d's high enough to monitor $[Ca^{2+}]_L$ within the range of 100's of μM ([68] for review).

Both techniques were used recently to measure $[Ca^{2+}]_L$ in neurones and neurone-related cells. First, the endoplasmic reticulum-targeted aequorin technique was applied to a PC12 cell line [69]. Second, the low-affinity fluorescent probes Mag-Fura-2 and Fluo-3FF were used in primary cultured sensory and central neurones [21, 68, 70, 71]. Importantly, these dyes were combined with monitoring of membrane currents in the whole-cell mode, thus permitting direct correlation between transmembrane currents and intra-ER /cytosolic Ca^{2+} dynamics.

These experiments led to the first direct demonstration of Ca^{2+}-induced Ca^{2+} release triggered by Ca^{2+} entry through voltage-gated Ca^{2+} channels [21]. It was demonstrated that (i) brief applications of caffeine triggered a rapid transient fall in $[Ca^{2+}]_L$ and (ii) that Ca^{2+} currents triggered by depolarisation resulted in a transient decrease in $[Ca^{2+}]_L$, the latter being indicative of physiological CICR. This CICR in response to Ca^{2+} entry was blocked by 50 μM ryanodine and potentiated by 1 mM caffeine. Most importantly, these experiments revealed the linear relation between Ca^{2+} entry and the amplitude of $[Ca^{2+}]_L$, indicating the gradual activation of CICR in neurones under physiological conditions. Further investigations of $[Ca^{2+}]_L$ dynamics in nerve cells have shown the co-existence of IICR and CICR and demonstrated that neuronal ER acts as a single functional Ca^{2+} store [70, 71].

The functional availability of Ca^{2+} release ultimately depended on Ca^{2+} uptake into the store via SERCA pumps, as inhibition of the latter by thapsigargin resulted in substantial decrease in $[Ca^{2+}]_L$ and completely eliminated both CICR and IICR. Importantly, the rate of SERCA-mediated Ca^{2+} uptake is primarily regulated by the ER Ca^{2+} content, as depletion of the ER from releasable Ca^{2+} increased the rate of uptake from ~ 1 μM/s under the resting condition to 5 - 7 μM/s; as replenishment of the stores progressed the uptake rate diminished towards the resting level [21].

The ER Ca^{2+} homeostasis and neuronal death

Dysfunction of cellular Ca^{2+} homeostasis is intimately connected with neuronal cell function, regulation of neuronal survival and induction of neuronal death [72, 73]. For many years fluctuations in free Ca^{2+} concentration in the cytosol have been regarded as a major substrate for Ca^{2+} signalling. In CNS neurones excessive glutamate-induced Ca^{2+} influx results in numerous Ca^{2+}-regulated pathways becoming over-stimulated resulting in free radical production, lipid peroxidation, mitochondrial depolarisation and impaired ion channel function [74, 75]. However, dynamic changes in free Ca^{2+} concentration within the ER and alterations in ER Ca^{2+} homeostasis may be even more important in regulating cell function and cell survival [15, 25, 76, 77] and distortion of ER Ca^{2+} handling may trigger various forms of neurodegeneration/neuropathy [78]. Furthermore, changes in Ca^{2+} homeostasis are intimately involved in age-dependent changes in the CNS, particularly in the demise of synapses and loss of axons [79-83].

As mentioned above, ER lumen provides for both protein synthesis and post-translational proteins folding. The proper folding of proteins, during which they acquire their tertiary and quaternary structures, is controlled by several enzymatic systems including peptydil prolyl isomerases and glycosylation enzymes (glycosidases and mannosidases - see [10] for review), as well as by several families of chaperones [23], such as glucose regulated proteins (*grp78*, *grp94*, etc), lectin-like chaperones (calreticulin, calnexin and calmegin) and protein disulfide isomerases (PDI, ERp57). Any disturbances in correct protein folding leads to accumulation of unfolded proteins within the endoplasmic reticulum inducing a condition generally referred to as the endoplasmic reticulum stress response [18, 23].

The endoplasmic reticulum stress response has been described in two forms, the unfolded protein response, UPR, and the endoplasmic reticulum-overload response, EOR (see [18, 84] for review). Overall the UPR is characterised by an activation of the expression of endoplasmic reticulum resident chaperones and by overall suppression of protein synthesis. The second type of endoplasmic reticulum stress response, the EOR is manifested by an activation of the transcription factor NF-κB, which in turn induces transcription of numerous pro-inflammatory proteins, and cell-adhesion molecules [18].

Importantly, both UPR and EOR may be triggered not only by biochemical stress, but also by disturbance in the endoplasmic reticulum Ca^{2+} homeostasis. In particular this pathway was found to

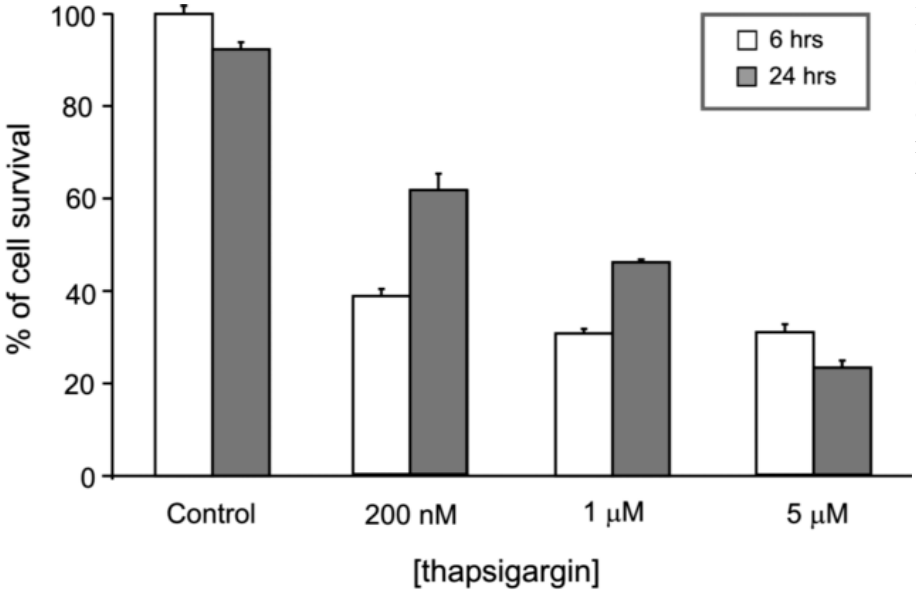

Fig. 2 Inhibition of SERCA-dependent ER uptake induces neuronal death. Survival of cultured dorsal root ganglion neurones exposed for 10 min to various concentrations of thapsigargin.

be operative in various types of neurones. The irreversible inhibition of SERCA by thapsigargin and hence constant depletion of the endoplasmic reticulum Ca^{2+} content effectively kills nerve cells (Fig. 2) and triggers a 200-fold increase in *grp78*, *grp94*, *gadd34* and *gadd153* in cultured cortical neurones [85], whereas the overall protein synthesis was inhibited [86], thus very much resembling the UPR. The EOR like response with a significant NF-κB activation was also shown in cortical neuronal cultures in response to endoplasmic reticulum depletion [87]. These observations fit together with the firmly established fact that treatment of neurones with thapsigargin triggers cell death by apoptotic mechanisms ([24, 26]. These findings naturally initiated the hypothesis that intra-endoplasmic reticulum Ca^{2+} content plays a critical role in the instigation of the endoplasmic reticulum stress response.

Indeed this hypothesis seems to be quite logical, especially remembering that at least one class of chaperones, the lectin-like ones, such as calreticulin, are the major Ca^{2+} binding proteins within the endoplasmic reticulum lumen, and $[Ca^{2+}]_L$ fluctuations inevitably affect them [23]. Interestingly also, the *grp78*, the chaperone which is up-regulated upon URP and plays a defensive role in fighting against the excess of unfolded proteins, also acts as a stabiliser of the endoplasmic reticulum Ca^{2+} content [88, 89]. Moreover, as the endoplasmic reticulum stress response induces expression not only of chaperones, it also up-regulates the expression of SERCA pumps [90] with the obvious aim of restoring the disturbed endoplasmic reticulum Ca^{2+} homeostasis.

ER Ca^{2+} homeostasis and brain ischemia

Disturbances in the ER Ca^{2+} homeostasis may also account for cell damage upon brain ischemia and excitotoxicity. This suggestion is based on several findings, produced by Japanese investigators [91-93]. First, they cloned ER resident chaperone, a 150-KDa oxygen-regulated protein (*orp150*) from astrocytes subjected to ischemic stress. The limited susceptibility of astrocytes to excitotoxicity and oxygen deprivation is well documented, and naturally it was suggested that *orp150* is somehow linked to it. More detailed investigations found that *orp150* is up-regulated in human brain subjected to a seizure attack and in mice hippocampus following kainate-induced lesion. Even more interestingly, hippocampal neurones isolated from *orp150* deficient mice responded by larger $[Ca^{2+}]_i$ increases when treated with glutamate or NMDA; this correlated with higher rates of glutamate-induced cell death. Furthermore, the survival of neurones from *orp150* deficient animals following kainate-induced brain lesions was significantly impaired. In contrast, increasing the levels of *orp150* in nerve cells by targeted overexpression, markedly improved neuronal survival after excitotoxic shock [91]. The glutamate-induced $[Ca^{2+}]_i$

loads were substantially reduced in cultured neurones with an increased level of *orp150*, thus demonstrating that this protein also stabilises cellular Ca^{2+} homeostasis, most likely by limiting Ca^{2+} release from the endoplasmic reticulum occurring upon excessive glutamate stimulation [91]. Finally, depletion of endoplasmic reticulum Ca^{2+} and consequent endoplasmic reticulum stress may participate in neurodegeneration during epileptic seizures, as the RyR blocker dantrolene had a significant neuroprotective action in various epilepsy models [94].

ER Ca^{2+} homeostasis and Alzheimer disease

The key role of disrupted ER Ca^{2+} homeostasis in the pathogenesis of AD has been suggested by several groups (see [27, 95, 96] for review), although the changes in the ER Ca^{2+} signalling in AD models have never been directly tested. The synthesis of a highly toxic isoform of amyloid beta peptide, Aβ(1-42), is controlled by presenilins, which are ER resident proteins [97]; and, moreover, the ER has been identified as a site of Aβ(1-42) synthesis. A direct role of the ER stress response in the pathogenesis of Alzheimer disease has been further substantiated by the recent observation that artificial expression of familial AD-specific mutations of presenilin-1 (PS-1) gene down-regulate the adaptive parts of the UPR, most notably by decreasing the levels of *grp78* [98, 99]. These observations gained pathophysiological significance after reduced levels of *grp78* were detected in the brains of familial AD patients [100]. Later it was found that an alternatively spliced form of presenilin 2 (PS-2) expressed in sporadic AD has the very same effect on the ER stress response [101]. Almost every manipulation with presenilins (either mutation in PS1 or PS2, or presenilin knockouts) resulted in the disruption of ER Ca^{2+} homeostasis [95]. As a general rule, overexpression of mutant forms of presenilins results in an increase in both IICR and CICR [102-104], whereas presenilin deficiency resulted in a decreased amplitude of Ca^{2+} release [95]. These findings led to the suggestion that presenilins might regulate the level of $[Ca^{2+}]_L$ and their up-regulation or expression of mutant forms (i.e. in AD-related pathology) results in an increase in intra-ER Ca^{2+} content. This particular suggestion was strengthened by the discovery that overexpression of Ca^{2+} binding proteins calbindin-D and calsenilin reversed the enhanced Ca^{2+} release in mutant PS1 and PS2 cells [104]. Furthermore, a store depletion-operated (capacitative) Ca^{2+} entry in cells expressing mutant PS1 or PS2 was enhanced [103, 105], whereas the genetic knockout of PS1, or the expression of an inactive form of PS1, potentiated this Ca^{2+} influx through the store-operated mechanism [105]. The latter results also suggest a role of presenilin in the regulation of $[Ca^{2+}]_L$ and it is interesting to note that the size of the $InsP_3$-mediated $[Ca^{2+}]_i$ responses in fibroblasts isolated from patients with high risk of family AD were much larger than those with a low risk of AD development [106]. These abnormalities in ER function could be detected much earlier than the onset of clinical symptoms, once more pointing to the possible aethiopathological role of ER Ca^{2+} dysregulation in the development of AD.

Conclusions

The ER Ca^{2+} homeostasis emerges as a main mechanism responsible for signal integration and processing within the endoplasmic reticulum. In physiological conditions, fluctuations of intra-ER free Ca^{2+} concentration link cellular activity with protein synthesis and post-translational modification. Disruption of the ER Ca^{2+} homeostasis triggers ER stress responses, which in turn may result in neurodegeneration and determine survival or death of neuronal cells.

Acknowledgements

This author's research was supported by BBSRC, Royal Society, and The Wellcome Trust.

References

1. **Berridge M. J., Lipp P. , Bootman M. D.** The versatility and universality of calcium signalling. *Nat. Rev. Mol. Cell. Biol.,* **1**: 11-21, 2000.
2. **Bootman M. D., Petersen O. H. , Verkhratsky A.** The endoplasmic reticulum is a focal point for co-ordination of cellular activity. *Cell Calcium,* **32**: 231-234, 2002.

3. **Kostyuk P. , Verkhratsky A.** Calcium stores in neurons and glia. *Neuroscience,* **63**: 381-404, 1994.
4. **Kostyuk P. G. , Verkhratsky A.** *Calcium signaling in the nervous system*. Chichester et al.: John Wiley & Sons, 1995.
5. **Verkhratsky A. J. , Petersen O. H.** Neuronal calcium stores. *Cell Calcium,* **24**: 333-343, 1998.
6. **Verkhratsky A. , Toescu E.C.** Integrative aspects of calcium signalling. New York, London: Plenum Press, 1998, p. 408.
7. **Petersen O. H., Petersen C. C. , Kasai H.** Calcium and hormone action. *Annu. Rev. Physiol.,* **56**: 297-319, 1994.
8. **Baumann O. , Walz B.** Endoplasmic reticulum of animal cells and its organization into structural and functional domains. *Int. Rev. Cytol.,* **205**: 149-214, 2001.
9. **Gerasimenko O. V., Gerasimenko J. V., Rizzuto R. R., Treiman M., Tepikin A. , Petersen O. H.** The distribution of the endoplasmic reticulum in living pancreatic acinar cells. *Cell Calcium,* **34**: 261-268, 2002.
10. **Chevet E., Cameron P. H., Pelletier M. F., Thomas D. Y. , Bergeron J. J.** The endoplasmic reticulum: integration of protein folding, quality control, signaling and degradation. *Curr. Opin. Struct. Biol.,* **11**: 120-124, 2001.
11. **Palade G.** Intracellular aspects of the process of protein synthesis. *Science,* **189**: 347-358, 1975.
12. **Nielsen S. P. , Petersen O. H.** Transport of calcium in the perfused submandibular gland of the cat. *J. Physiol.,* **223**: 685-697, 1972.
13. **Ford L. E. , Podolsky R. J.** Regenerative calcium release within muscle cells. *Science,* **167**: 58-59, 1970.
14. **Berridge M. J.** The endoplasmic reticulum: a multifunctional signalling organelle. *Cell Calcium,* **32**: 235-249, 2002.
15. **Verkhratsky A. , Petersen O. H.** The endoplasmic reticulum as an integrating signalling organelle: from neuronal signalling to neuronal death. *Eur. J. Pharmacol.,* **447**: 141-154, 2002.
16. **Bootman M. D., Lipp P. , Berridge M. J.** The organisation and functions of local Ca^{2+} signals. *J. Cell. Sci.,* **114**: 2213-2222, 2001.
17. **Mogami H., Nakano K., Tepikin A. V. , Petersen O. H.** Ca^{2+} flow via tunnels in polarized cells: recharging of apical Ca^{2+} stores by focal Ca^{2+} entry through basal membrane patch. *Cell,* **88**: 49-55, 1997.
18. **Pahl H. L.** Signal transduction from the endoplasmic reticulum to the cell nucleus. *Physiol. Rev.,* **79**: 683-701, 1999.
19. **Chapman R., Sidrauski C. , Walter P.** Intracellular signaling from the endoplasmic reticulum to the nucleus. *Annu. Rev. Cell Dev. Biol.,* **14**: 459-485, 1998.
20. **Verkhratsky A.** The endoplasmic reticulum and neuronal calcium signalling. *Cell Calcium,* **32**: 393-404, 2002.
21. **Solovyova N., Veselovsky N., Toescu E.C., Verkhratsky A.** Ca^{2+} dynamics in the lumen of the endoplasmic reticulum in sensory neurones: direct visualisation of Ca^{2+}-induced Ca^{2+} release triggered by physiological Ca^{2+} entry. *EMBO J.,* **21**: 622-630, 2002.
22. **Camello C., Lomax R., Petersen O. H. , Tepikin A. V.** Calcium leak from intracellular stores—the enigma of calcium signalling. *Cell Calcium,* **34**: 355-361, 2002.
23. **Michalak M., Robert Parker J. M. , Opas M.** Ca^{2+} signaling and calcium binding chaperones of the endoplasmic reticulum. *Cell Calcium,* **32**: 269-278, 2002.
24. **Paschen W.** Dependence of vital cell function on endoplasmic reticulum calcium levels: implications for the mechanisms underlying neuronal cell injury in different pathological states. *Cell Calcium,* **29**: 1-11, 2001.
25. **Paschen W.** Mechanisms of neuronal cell death: diverse roles of calcium in the various subcellular compartments. *Cell Calcium,* **34**: 305-310, 2003.
26. **Paschen W.** Endoplasmic reticulum: a primary target in various acute disorders and degenerative diseases of the brain. *Cell Calcium,* **34**: 365-383, 2003.
27. **Toescu E. C., Verkhratsky A.** Neuronal ageing from an intraneuronal perspective: roles of endoplasmic reticulum and mitochondria. *Cell Calcium,* **34**: 311-323, 2003.
28. **Wuytack F., Raeymaekers L. , Missiaen L.** Molecular physiology of the SERCA and SPCA pumps. *Cell Calcium,* **32**: 279-305, 2002.
29. **Meldolesi J.** Rapidly exchanging Ca^{2+} stores in neurons: molecular, structural and functional properties. *Prog. Neurobiol.,* **65**: 309-338, 2001.
30. **Galione A. , Churchill G. C.** Interactions between calcium release pathways: multiple messengers and multiple stores. *Cell Calcium,* **32**: 343-354, 2002.
31. **Rossi D. , Sorrentino V.** Molecular genetics of ryanodine receptors Ca^{2+} release channels. *Cell Calcium,* **32**: 307-319, 2002.
32. **Taylor C. W. , Laude A. J.** IP3 receptors and their regulation by calmodulin and cytosolic Ca^{2+}. *Cell Calcium,* **32**: 321-334, 2002.
33. **Young K. W. , Nahorski S. R.** Sphingosine 1-phosphate: a Ca^{2+} release mediator in the balance. *Cell Calcium,* **34**: 335-341, 2002.
34. **Bak J., White P., Timar G., Missiaen L., Genazzani A. A. , Galione A.** Nicotinic acid adenine dinucleotide phosphate triggers Ca^{2+} release from brain microsomes. *Curr. Biol.,* **9**: 751-754, 1999.
35. **Chameau P., Van de Vrede Y., Fossier P. , Baux G.** Ryanodine-, IP3- and NAADP-dependent calcium stores control acetylcholine release. *Pflugers Arch.,* **443**: 289-296, 2001.
36. **Kuba K.** Release of calcium ions linked to the activation of potassium conductance in a caffeine-treated sympathetic neurone. *J. Physiol.,* **298**: 251-269, 1980.
37. **Morita K., Koketsu K. , Kuba K.** Oscillation of $[Ca^{2+}]_i$-linked K^+ conductance in bullfrog sympathetic ganglion cell is sensitive to intracellular anions. *Nature,* **283**: 204-205, 1980.
38. **Brown D. A., Constanti A. , Adams P. R.** Ca-activated potassium current in vertebrate sympathetic neurons. *Cell Calcium,* **4**: 407-420, 1983.
39. **Salanki J., Budai D. , Vero M.** Modification of bursting in a Helix neuron by drugs influencing intracellular regulation of calcium level. *Acta Physiol. Hung.,* **62**: 213-224, 1983.
40. **Tsien R. , Pozzan T.** Measurement of cytosolic free Ca^{2+} with quin2. *Meth. Enzymol.,* **172**: 230-262, 1989.
41. **Neering I. R. , McBurney R. N.** Role for microsomal Ca storage in mammalian neurones? *Nature,* **309**: 158-160, 1984.

42. **Lipscombe D., Madison D. V., Poenie M., Reuter H., Tsien R. W. , Tsien R. Y.** Imaging of cytosolic Ca^{2+} transients arising from Ca^{2+} stores and Ca^{2+} channels in sympathetic neurons. *Neuron,* **1**: 355-365, 1988.
43. **Marrion N. V. , Adams P. R.** Release of intracellular calcium and modulation of membrane currents by caffeine in bull-frog sympathetic neurones. *J. Physiol.,* **445**: 515-535, 1992.
44. **Thayer S. A., Hirning L. D. , Miller R. J.** The role of caffeine-sensitive calcium stores in the regulation of the intracellular free calcium concentration in rat sympathetic neurons *in vitro*. *Mol. Pharmacol.,* **34**: 664-673, 1988.
45. **Friel D. D. , Tsien R. W.** 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, 1992.
46. **Usachev Y., Shmigol A., Pronchuk N., Kostyuk P. , Verkhratsky A.** Caffeine-induced calcium release from internal stores in cultured rat sensory neurons. *Neuroscience,* **57**: 845-859, 1993.
47. **Usachev Y., Kostyuk P. , Verkhratsky A.** 3-Isobutyl-1-methylxanthine (IBMX) affects potassium permeability in rat sensory neurones via pathways that are sensitive and insensitive to $[Ca^{2+}]_{in}$. *Pflugers Arch.,* **430**: 420-428, 1995.
48. **Verkhratsky A. , Shmigol A.** Calcium-induced calcium release in neurones. *Cell Calcium* **19**: 1-14, 1996.
49. **Bleakman D., Roback J. D., Wainer B. H., Miller R. J., Harrison N. L.** Calcium homeostasis in rat septal neurons in tissue culture. *Brain Res.,* **600**: 257-267, 1993.
50. **Shmigol A., Kirischuk S., Kostyuk P. , Verkhratsky A.** Different properties of caffeine-sensitive Ca^{2+} stores in peripheral and central mammalian neurones. *Pflugers Arch.,* **426**: 174-176, 1994.
51. **Shmigol A., Kostyuk P. , Verkhratsky A.** Role of caffeine-sensitive Ca^{2+} stores in Ca^{2+} signal termination in adult mouse DRG neurones. *Neuroreport,* **5**: 2073-2076, 1994.
52. **Shmigol A., Kostyuk P. , Verkhratsky A.** Dual action of thapsigargin on calcium mobilization in sensory neurons: inhibition of Ca^{2+} uptake by caffeine-sensitive pools and blockade of plasmalemmal Ca^{2+} channels. *Neuroscience,* **65**: 1109-1118, 1995.
53. **Shmigol A., Svichar N., Kostyuk P. , Verkhratsky A.** Gradual caffeine-induced Ca^{2+} release in mouse dorsal root ganglion neurons is controlled by cytoplasmic and luminal Ca^{2+}. *Neuroscience,* **73**: 1061-1067, 1996.
54. **Shmigol A., Verkhratsky A. , Isenberg G.** Calcium-induced calcium release in rat sensory neurons. *J. Physiol. Lond.,* **489**: 627-636, 1995.
55. **Kirischuk S., Voitenko N., Kostyuk P. , Verkhratsky A.** Calcium signalling in granule neurones studied in cerebellar slices. *Cell Calcium,* **19**: 59-71, 1996.
56. **Kano M., Garaschuk O., Verkhratsky A. , Konnerth A.** Ryanodine receptor-mediated intracellular calcium release in rat cerebellar Purkinje neurones. *J. Physiol.,* **487**: 1-16, 1995.
57. **Garaschuk O., Yaari Y. , Konnerth A.** Release and sequestration of calcium by ryanodine-sensitive stores in rat hippocampal neurones. *J. Physiol.,* **502**: 13-30, 1997.
58. **Pfaffinger P. J., Leibowitz M. D., Subers E. M., Nathanson N. M., Almers W., Hille B.** Agonists that suppress M-current elicit phosphoinositide turnover and Ca^{2+} transients, but these events do not explain M-current suppression. *Neuron,* **1**: 477-484, 1988.
59. **Otsu H., Yamamoto A., Maeda N., Mikoshiba K. , Tashiro Y.** Immunogold localization of inositol 1, 4, 5-trisphosphate (InsP3) receptor in mouse cerebellar Purkinje cells using three monoclonal antibodies. *Cell Struct. Funct.,* **15**: 163-173, 1990.
60. **Walton P. D., Airey J. A., Sutko J. L., Beck C. F., Mignery G. A., Sudhof T. C., Deerinck T. J. , Ellisman M. H.** Ryanodine and inositol trisphosphate receptors coexist in avian cerebellar Purkinje neurons. *J. Cell Biol.,* **113**: 1145-1157, 1991.
61. **Khodakhah K. , Ogden D.** Functional heterogeneity of calcium release by inositol trisphosphate in single Purkinje neurones, cultured cerebellar astrocytes, and peripheral tissues. *Proc. Natl. Acad. Sci. USA,* **90**: 4976-4980, 1993.
62. **Khodakhah K., Ogden D.** Fast activation and inactivation of inositol trisphosphate-evoked Ca^{2+} release in rat cerebellar Purkinje neurones. *J. Physiol.,* **487**: 343-358, 1995.
63. **Svichar N., Shmigol A., Verkhratsky A. , Kostyuk P.** $InsP_3$-induced Ca^{2+} release in dorsal root ganglion neurones. *Neurosci. Lett.,* **227**: 107-110, 1997.
64. **Svichar N., Shmigol A., Verkhratsky A. , Kostyuk P.** ATP induces Ca^{2+} release from IP_3-sensitive Ca^{2+} stores exclusively in large DRG neurones. *Neuroreport,* **8**: 1555-1559, 1997.
65. **Kirischuk S., Matiash V., Kulik A., Voitenko N., Kostyuk P. , Verkhratsky A.** Activation of P2-purino-, α1-adreno and H1-histamine receptors triggers cytoplasmic calcium signalling in cerebellar Purkinje neurons. *Neuroscience,* **73**: 643-647, 1996.
66. **Alvarez J. , Montero M.** Measuring $[Ca^{2+}]$ in the endoplasmic reticulum with aequorin. *Cell Calcium,* **32**: 251-260, 2002.
67. **Demaurex N. , Frieden M.** Measurements of the free luminal ER Ca^{2+} concentration with targeted "cameleon" fluorescent proteins. *Cell Calcium,* **34**: 109-119, 2003.
68. **Solovyova N. , Verkhratsky A.** Monitoring of free calcium in the neuronal endoplasmic reticulum: An overview of modern approaches. *J. Neurosci. Meth.,* **122:** 1-12, 2002.
69. **Alonso M. T., Barrero M. J., Michelena P., Carnicero E., Cuchillo I., Garcia A. G., Garcia-Sancho J., Montero M. , Alvarez J.** Ca^{2+}-induced Ca^{2+} release in chromaffin cells seen from inside the ER with targeted aequorin. *J. Cell Biol.,* **144**: 241-254, 1999.
70. **Solovyova N., Fernyhough P., Glazner G. , Verkhratsky A.** Xestospongin C empties the ER calcium store but does not inhibit $InsP_3$-induced Ca^{2+} release in cultured dorsal root ganglia neurones. *Cell Calcium,* **32**: 49-52, 2002.
71. **Solovyova N. , Verkhratsky A.** Neuronal endoplasmic reticulum acts as a single functional Ca^{2+} store shared by ryanodine and inositol-1,4,5-trisphosphate receptors as revealed by intra-ER $[Ca^{2+}]$ recordings in single rat sensory neurones. *Pflugers Arch.,* **446**: 447-454, 2003.

72. **Leist M., Nicotera P.** Calcium and neuronal death. *Rev. Physiol. Biochem. Pharmacol.,* **132**: 79-125, 1998.
73. **Sattler R., Tymianski M.** Molecular mechanisms of calcium-dependent excitotoxicity. *J. Mol. Med.* **78**: 3-13, 2000.
74. **Mattson M. P.** Modification of ion homeostasis by lipid peroxidation: roles in neuronal degeneration and adaptive plasticity. *Trends Neurosci.,* **21**: 53-57, 1998.
75. **Lu C., Chan S. L., Fu W., Mattson M. P.** The lipid peroxidation product 4-hydroxynonenal facilitates opening of voltage-dependent Ca^{2+} channels in neurons by increasing protein tyrosine phosphorylation. *J. Biol. Chem.*, 2002.
76. **Glazner G., Fernyhough P.** Neuronal survival in the balance: are endoplasmic reticulum membrane proteins the fulcrum? *Cell Calcium,* **32**: 421-433, 2002.
77. **Mattson M. P., Gary D. S., Chan S. L., Duan W.** Perturbed endoplasmic reticulum function, synaptic apoptosis and the pathogenesis of Alzheimer's disease. *Biochem. Soc. Symp.*, 151-162, 2001.
78. **Huang T. J., Sayers N. M., Fernyhough P., Verkhratsky A.** 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, 2002.
79. **Kirischuk S., Verkhratsky A.** Calcium homeostasis in aged neurones. *Life Sci.,* **59**: 451-459, 1996.
80. **Kirischuk S., Voitenko N., Kostyuk P., Verkhratsky A.** Age-associated changes of cytoplasmic calcium homeostasis in cerebellar granule neurons in situ: investigation on thin cerebellar slices. *Exp. Gerontol.,* **31**: 475-487, 1996.
81. **Verkhratsky A., Shmigol A., Kirischuk S., Pronchuk N., Kostyuk P.** Age-dependent changes in calcium currents and calcium homeostasis in mammalian neurons. *Ann. NY Acad. Sci.,* **747**: 365-381, 1994.
82. **Verkhratsky A., Toescu E. C.** Calcium and neuronal ageing. *Trends Neurosci.,* **21**: 2-7, 1998.
83. **Xiong J., Verkhratsky A., Toescu E. C.** Changes in mitochondrial status associated with altered Ca^{2+} homeostasis in aged cerebellar granule neurons in brain slices. *J. Neurosci.,* **22**: 10761-10771, 2002.
84. **Patil C., Walter P.** Intracellular signaling from the endoplasmic reticulum to the nucleus: the unfolded protein response in yeast and mammals. *Curr. Opin. Cell Biol.,* **13**: 349-355, 2001.
85. **Mengesdorf T., Althausen S., Oberndorfer I. , Paschen W.** Response of neurons to an irreversible inhibition of endoplasmic reticulum Ca^{2+}-ATPase: relationship between global protein synthesis and expression and translation of individual genes. *Biochem. J.,* **356**: 805-812, 2001.
86. **Doutheil J., Treiman M., Oschlies U. , Paschen W.** Recovery of neuronal protein synthesis after irreversible inhibition of the endoplasmic reticulum calcium pump. *Cell Calcium,* **25**: 419-428, 1999.
87. **Glazner G. W., Camandola S., Geiger J. D. , Mattson M. P.** 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, 2001.
88. **Lee J., Bruce-Keller A. J., Kruman Y., Chan S. L. , Mattson M. P.** 2-Deoxy-D-glucose protects hippocampal neurons against excitotoxic and oxidative injury: evidence for the involvement of stress proteins. *J. Neurosci. Res.,* **57**: 48-61, 1999.
89. **Yu Z., Luo H., Fu W. , Mattson M. P.** 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, 1999.
90. **Thuerauf D. J., Hoover H., Meller J., Hernandez J., Su L., Andrews C., Dillmann W. H., McDonough P. M., Glembotski C. C.** Sarco/endoplasmic reticulum calcium ATPase-2 expression is regulated by ATF6 during the endoplasmic reticulum stress response. *J. Biol. Chem.,* **276**: 48309-48317, 2001.
91. **Kitao Y., Ozawa K., Miyazaki M., Tamatani M., Kobayashi T., Yanagi H., Okabe M., Ikawa M., Yamashima T., Stern D. M., Hori O. , Ogawa S.** Expression of the endoplasmic reticulum molecular chaperone (ORP150) rescues hippocampal neurons from glutamate toxicity. *J. Clin. Invest.,* **108**: 1439-1450, 2001.
92. **Kuwabara K., Matsumoto M., Ikeda J., Hori O., Ogawa S., Maeda Y., Kitagawa K., Imuta N., Kinoshita T., Stern D. M., Yanagi H. , Kamada T.** Purification and characterization of a novel stress protein, the 150-kDa oxygen-regulated protein (ORP150), from cultured rat astrocytes and its expression in ischemic mouse brain. *J. Biol. Chem.,* **271**: 5025-5032, 1996.
93. **Ozawa K., Kuwabara K., Tamatani M., Takatsuji K., Tsukamoto Y., Kaneda S., Yanagi H., Stern D. M., Eguchi Y., Tsujimoto Y., Ogawa S. , Tohyama M.** 150-kDa oxygen-regulated protein (ORP150) suppresses hypoxia-induced apoptotic cell death. *J. Biol. Chem.,* **274**: 6397-6404, 1999.
94. **Niebauer M., Gruenthal M.** Neuroprotective effects of early vs. late administration of dantrolene in experimental status epilepticus. *Neuropharmacology,* **38**: 1343-1348, 1999.
95. **LaFerla F. M.** Calcium dyshomeostasis and intracellular signalling in Alzheimer disease. *Nat. Rev. Neurosci.,* **3**: 862-872, 2002.
96. **Mattson M. P. , Chan S. L.** Neuronal and glial calcium signaling in Alzheimer's disease. *Cell Calcium,* **34**: 385-397, 2003.
97. **Refolo L. M., Eckman C., Prada C. M., Yager D., Sambamurti K., Mehta N., Hardy J. , Younkin S. G.** Antisense-induced reduction of presenilin 1 expression selectively increases the production of amyloid beta42 in transfected cells. *J. Neurochem.,* **73**: 2383-2388, 1999.
98. **Imaizumi K., Katayama T. , Tohyama M.** Presenilin and the UPR. *Nat. Cell. Biol.,* **3**: E104, 2001.
99. **Katayama T., Imaizumi K., Honda A., Yoneda T., Kudo T., Takeda M., Mori K., Rozmahel R., Fraser P., George-Hyslop P. S. , Tohyama M.** Disturbed activation of endoplasmic reticulum stress transducers by familial Alzheimer's disease-linked presenilin-1 mutations. *J. Biol. Chem.,* **276**: 43446-43454, 2001.
100. **Katayama T., Imaizumi K., Sato N., Miyoshi K., Kudo T., Hitomi J., Morihara T., Yoneda T., Gomi F., Mori Y., Nakano Y., Takeda J., Tsuda T., Itoyama Y., Murayama O., Takashima A., St George-Hyslop P.,**

Takeda M. , Tohyama M. Presenilin-1 mutations down-regulate the signalling pathway of the unfolded-protein response. *Nat. Cell. Biol.,* **1**: 479-485, 1999.

101. **Sato N., Imaizumi K., Manabe T., Taniguchi M., Hitomi J., Katayama T., Yoneda T., Morihara T., Yasuda Y., Takagi T., Kudo T., Tsuda T., Itoyama Y., Makifuchi T., Fraser P. E., St George-Hyslop P., Tohyama M.** Increased production of beta-amyloid and vulnerability to endoplasmic reticulum stress by an aberrant spliced form of presenilin 2. *J. Biol. Chem.,* **276**: 2108-2114, 2001.
102. **Chan S. L., Mayne M., Holden C. P., Geiger J. D. , Mattson M. P.** Presenilin-1 mutations increase levels of ryanodine receptors and calcium release in PC12 cells and cortical neurons. *J. Biol. Chem.,* **275**: 18195-18200, 2000.
103. **Leissring M. A., Akbari Y., Fanger C. M., Cahalan M. D., Mattson M. P. , LaFerla F. M.** Capacitative calcium entry deficits and elevated luminal calcium content in mutant presenilin-1 knockin mice. *J. Cell. Biol.,* **149**: 793-798, 2000.
104. **Leissring M. A., Yamasaki T. R., Wasco W., Buxbaum J. D., Parker I. , LaFerla F. M.** Calsenilin reverses presenilin-mediated enhancement of calcium signaling. *Proc. Natl. Acad. Sci. USA,* **97**: 8590-8593, 2000.
105. **Yoo A. S., Cheng I., Chung S., Grenfell T. Z., Lee H., Pack-Chung E., Handler M., Shen J., Xia W., Tesco G., Saunders A. J., Ding K., Frosch M. P., Tanzi R. E. , Kim T. W.** Presenilin-mediated modulation of capacitative calcium entry. *Neuron,* **27**: 561-572, 2000.
106. **Etcheberrigaray R., Hirashima N., Nee L., Prince J., Govoni S., Racchi M., Tanzi R. E., Alkon D. L.** Calcium responses in fibroblasts from asymptomatic members of Alzheimer's disease families. *Neurobiol. Dis.,* **5**: 37-45, 1998.