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MINI-REVIEW

Endoplasmic reticulum dysfunction – a common denominator for cell injury in acute and degenerative diseases of the brain?

Wulf Paschen* and Aase Frandsen†

**Department of Experimental Neurology, Max-Planck-Institute for Neurological Research, Cologne, Germany*

†Department of Pharmacology, The Royal Danish School of Pharmacy, Copenhagen, Denmark

Abstract

Various physiological, biochemical and molecular biological disturbances have been put forward as mediators of neuronal cell injury in acute and chronic pathological states of the brain such as ischemia, epileptic seizures and Alzheimer's or Parkinson's disease. These include over-activation of glutamate receptors, a rise in cytoplasmic calcium activity and mitochondrial dysfunction. The possible involvement of the endoplasmic reticulum (ER) dysfunction in this process has been largely neglected until recently, although the ER plays a central role in important cell functions. Not only is the ER involved in the control of cellular calcium homeostasis, it is also the subcellular compartment in which the folding and processing of membrane and secretory proteins takes place. The fact that blocking of these processes is sufficient to cause cell damage indicates that they are crucial for normal cell functioning. This review presents evidence that ER function is disturbed in many acute and chronic diseases of the brain. The complex processes taken place in this subcellular compartment are however, affected in different ways in various disorders; whereas the ER-associated degradation of misfolded proteins is affected in Parkinson's disease, it is the unfolded protein response which is down-regulated in Alzheimer's disease and the ER calcium homeostasis that is disturbed in ischemia. Studying the consequences of the observed deteriorations of ER function and identifying the mechanisms causing ER dysfunction in these pathological states of the brain will help to elucidate whether neurodegeneration is indeed caused by these disturbances, and will help to fascilitate the search for drugs capable of blocking the pathological process directly at an early stage.

Keywords: Alzheimer's disease, endoplasmic reticulum, ischemia, neuronal cell injury, Parkinson's disease, unfolded protein response.

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The endoplasmic reticulum (ER) is an intracellular compartment involved in calcium storage and calcium signalling. Furthermore, all newly synthesized membrane and secretory proteins are folded and processed there. These functions are strictly calcium dependent and to carry them out correctly the ER requires a level of calcium calcium activity almost as high as that of the extracellular space, high levels of the enzymes involved in the folding and processing reactions, and an oxidative environment. A central role is played by the chaperone GRP78, as indicated by the observations that overexpression of GRP78 protect cells from death associated with ER stress whereas down-regulation of GRP78 increase the sensitivity of cells to ER stress (for a review see Kaufman 1999).

Conditions associated with ER dysfunction induce highly conserved stress responses, the unfolded-protein response (UPR), the ER overload response (EOR) and the ER-associated degradation (ERAD) (for a review see Kaufman 1999). UPR has been elucidated in yeast cells. It is characterized by activation of the double-stranded RNA-activated protein kinase (PKR) and the PKR-like ER kinase (PERK), protein kinases, which then suppress the initiation step of protein synthesis, and by activation of Ire1p, which induces the expression of genes coding for ER-resident stress proteins. PERK and Ire1p are ER-resident protein kinase which are activated by autophosphorylation. After activation, PERK induces the phosphorylation of the eukaryotic initiation factor eIF-2α thereby blocking the initiation step of protein

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Correspondence and reprint requests to Prof. Wulf Paschen, Department of Experimental Neurology, Max-Planck-Institute for Neurological Research, Gleuelerstr. 50, 50931 Cologne, Germany.
E-mail: paschen@mpin-koeln.mpg.de

Abbreviations used: ER, endoplasmic reticulum; ERAD, ER-associated degradation; EOR, ER overload response; FAD, familial Alzheimer's disease; ORP150, oxygen-regulated protein; PKR, double-stranded RNA-activated protein kinase; PERK, PKR-like ER kinase; SERP1, stress-associated endoplasmic reticulum protein 1; SAD, sporadic Alzheimer's disease; SR, sarcoplasmic reticulum; UPR, unfolded-protein response;

synthesis. Activated Ire1p, on the other hand, leads to the splicing of HAC1 mRNA, which after splicing is translated into the HAC1 protein (HAC1p). HAC1p is a transcription factor which induces the expression of UPR genes by binding to the unfolded protein response element in the promotor of the respective genes. EOR is characterized by an activation of the transcription factor NF-κB, leading to an up-regulation of the expression of target genes encoding pro-inflammatory proteins. ERAD triggers the ubiquitination of misfolded proteins accumulating in the lumen of the ER, and the degradation of poly-ubiquinated proteins at the proteasome.

The ER has close physical and functional contacts to both the cell membrane and to mitochondria (for a recent review see Putney and Ribeiro 2000; Rutter and Rizzuto 2000). For instance, ER calcium release triggered under conditions associated with cell activation induces mitochondrial ATP synthesis. These interactions between organelles are essential for correct cell functioning under physiological conditions. Under pathological conditions, however, an apoptotic cross talk has been identified between ER and mitochondria (Häcki *et al*. 2000). These authors have shown that conditions associated with disturbed ER functions induce release of cytochrome c from mitochondria and activation of caspase 3, pathological processes now seen as hallmarks of programmed cell death. Mitochondrial cytochrome c release and activation of caspase 3 have both been shown to be completely suppressed in cells overexpressing Bcl-2 targeted specifically to the ER, implying that in pathological cerebral states triggering programmed cell death stress-induced ER dysfunction may be a process up-stream of mitochondrial dysfunction.

The notion that ER dysfunction may be involved in the pathogenesis of neuronal cell injury after a severe form of stress was first put forward in 1996 (Paschen 1996). Under pathological conditions various mechanisms could disturb ER functions: A severe insult could have a direct effect on ER calcium homeostasis, e.g. by inactivating ER calcium pump, leading to a blocking of the folding and processing reactions and an accumulation of unfolded proteins in the ER lumen. Alternatively, it could interfere with the folding and processing reactions directly. Owing to the wide-spread acceptance of the traditional calcium hypothesis (Siesjö 1981), changes in cytoplasmic calcium homeostasis are generally believed to be the central pathomechanism even under conditions associated with disturbances of ER calcium homeostasis. But it has since been proposed that disturbance of ER function may be sufficient to induce cell injury, and that depletion of ER calcium stores even without changes in cytoplasmic calcium activity may activate the process of programed cell death (for a comprehensive discussion see Paschen and Doutheil 1999). The present review summarizes new observations pointing to a role of ER dysfunction in the development of neuronal cell injury in acute and chronic diseases of the brain, including ischemia, epileptic seizures, and Alzheimer's or Parkinson disease.

ER dysfunction in ischemia and epileptic seizures

Arguments for a role of ER dysfunction in the development of ischemic neuronal injury have been summarized recently (Paschen and Doutheil 1999). The notion that disturbances of ER function may play a key role in the pathogenesis of neuronal cell injury after transient global cerebral ischemia rose from the observation that the neuronal stress response triggered by transient ischemia is in many respects similar to that induced by conditions associated with ER dysfunction (Paschen 1996). Further evidence to support this hypothesis has since accumulated: For instance, the observation that cerebral ischemia causes an activation of eIF-2α kinase PERK (Kumar *et al*. 2001), an ER resident protein kinase which is specifically activated under conditions of ER dysfunction (Harding *et al*. 1999), suggests that ER dysfunction is linked to ischemically induced suppression of protein synthesis. Protein aggregates found after transient cerebral ischemia indicate that the folding reaction is disturbed under such conditions (Hu *et al*. 2000). However, whether these aggregates are the consequence of ischemia-induced ER dysfunction still remains to be established. Ischemia also inhibits calcium uptake into brain microsomes (Parsons *et al*. 1997). The mechanisms underlying ischemia-induced ER dysfunction have not been fully elucidated but there is some evidence that NO may be involved (Doutheil *et al*. 2000). After transient ischemia, ER calcium stores have been shown to be depleted in vulnerable neurons, and in these experiments restoration of ER calcium stores was only observed in animals pre-treated with an NO synthase inhibitor (Kohno *et al*. 1997). Furthermore, in-vitro studies indicate that exposure of neurons to NO causes inactivation of ER Ca^{2+} ATPase, depletion of ER calcium stores and suppression of protein synthesis, i.e. changes similar to those induced by transient cerebral ischemia (Doutheil *et al*. 2000).

Activation of the expression of the stress-associated endoplasmic reticulum protein 1 (SERP1) in the penumbral region surrounding the ischemic focus in middle cerebral artery occlusion in rats suggests that ER dysfunction also plays a role in focal cerebral ischemia (Yamaguchi *et al*. 1999). The size of infarcts is indeed markedly reduced in mice with targeted overexpression of oxygen-regulated protein (ORP150), a novel endoplasmic reticulum chaperone (Tamatani *et al*. 2001). The mechanisms underlying induction of ER dysfunction in focal cerebral ischemia remains to be established. Besides nitric oxide (see above), spreading depression (Iijima *et al*. 1992) and acidic and alkaline pH-shifts observed in the penumbra surrounding the ischemic territory (Back *et al*. 2000) may contribute to the development of ER dysfunction. Spreading depression induces a transient decline in ATP levels (Mies and Paschen 1984) which may be sufficient to deplete ER calcium stores, as it has been shown that relatively small reductions in ATP levels are

sufficient to induce severe loss of calcium from ER stores (Kahlert and Reiser 2000). Furthermore, ER calcium stores are depleted under conditions associated with an acidic or alkaline pH-shift (Kimura *et al*. 2000; Willoughby *et al*. 2001).

Studies set up to investigate the signal transduction pathways involved in ischemic cell injury of the brain are usually focused on mechanisms underlying the pathogenesis of neuronal cell injury. However, disturbances of the functioning of endothelial and smooth-muscle cells, which critically affect the regulation of cerebral blood vessel tonus may also contribute to the pathological process of stroke. After transient cerebral ischemia, the regulation of cerebral blood flow is impaired, leading to postischemic hypoperfusion (for a review see Hossmann 1997). Ischemically induced disturbances of the functioning of the sarcoplasmic reticulum (SR) of cerebral blood vessels may contribute to this process. It has been shown that in adult cerebral arteries contractility depends on a functional SR compartment (Long *et al*. 2000), that functional elimination of the SR compartment causes contraction of cerebral arteries (Asano *et al*. 1996), and that hypoxia followed by reoxygenation impairs aortic vasoconstrictor responses, which in turn are dependent on SR calcium release (Gao *et al*. 1996). This suggests that transient cerebral ischemia could induce a long-lasting disturbance of SR calcium homeostasis of cerebral arteries.

The calcium store depletion of ER is also believed to play a role in the pathogenesis of neuronal cell injury in status epilepticus, as indicated by the observation that dantrolene, a blocker of the ER ryanodine receptor, is neuroprotective in models of epileptic seizures (Berg *et al*. 1995; Niebauer and Gruenthal 1999). Furthermore, the seizure-inducing drug bicuculline has been shown to trigger calcium-induced calcium release from ER stores (Mestdagh and Wülfert 1999), while the microsomal Ca^{2+} ATPase was found to be inhibited in pilocarpine-induced status epilepticus (Parson *et al*. 2000).

ER dysfunction in Alzheimer's and Parkinson's disease

The hypothesis that ER dysfunction plays a major role in the development of neuronal cell injury in Alzheimer's disease has recently been put forward by Katayama *et al.* (1999). The notion is based on the observation that mutations in the presenilin-1 gene, causing early onset familial Alzheimer's disease (FAD), down-regulate the signalling pathway of UPR. Expression of mutant presenilins induced a decrease in GRP78 protein levels and an increase in the sensitivity of cells to conditions associated with ER stress (Katayama *et al*. 1999). This pathological process was completely blocked in cells over-expressing grp78 (Katayama *et al*. 1999). It has been suggested that mutations in the presenilin-1 gene disrupt the action of IRE1, an ER-membran protein which plays a central role in the signalling pathway activated under conditions of ER dysfunction. Phosphorylation of Ire1p is responsible for the activation of the expression of genes coding for ER-resident stress proteins. The authors did indeed find a marked decrease of GRP78 protein levels in the brains of FAD patients (Katayama *et al*. 1999). Furthermore, it has been shown that activation of UPR leads to proteolytic cleavage of IRE1, and accumulation of IRE1 fragments in the nucleus, and that this process is disturbed in presenilin-1 knock-out cells (Niwa *et al*. 1999). The role played by IRE1 in the signalling pathway of UPR is indicated by the observation that under conditions of ER stress the apoptotic process is markedly increased in cells expressing a dominant-negative form of IRE1 (Miyoshi *et al*. 2000). However, the precise role of presenilins in the induction of UPR, and the consequence of presenilin mutations on UPR are still a matter of debate (Sato *et al*. 2000; Imaizumi *et al*. 2001).

The exact molecular mechanisms underlying the development of neuronal cell injury in sporadic Alzheimer's disease (SAD) are less well understood. A novel presenilin-2 splice variant has been found in the brains of SAD patients (Sato *et al*. 1999). This new splice variant is also produced under hypoxic conditions, and cells expressing this splice variant are more vulnerable to stress conditions such as hypoxia, tunicamycine or calcium ionophore exposure (Sato *et al*. 1999). Recently, this presenilin-2 splice variant has been shown to cause significant increases in Aβ40 and Aβ42 production, and also to interfere with the signaling pathway of UPR by blocking IRE1p phosphorylation, and to increase the sensitivity of cells to conditions associated with ER stress (Sato *et al*. 2001).

Another mutation which has been identified in the brains of Alzheimer's disease and Down's syndrom patients is a frameshift of ubiquitin-B, leading to ubiquitin-B aberrant in the carboxyl terminus (Ub^{+1}; van Leeuwen *et al*. 1998). It has been shown that Ub^{+1} is an efficient substrate for polyubiquitination and that the resulting polyubiqitinated chains potentially inhibit the proteasomes (Lam *et al*. 2000). Proteasomes play a central role in the degradation of misfolded proteins accumulating in the lumen of the ER. Under conditions of ER stress, endoplasmic reticulum-associated degradation (ERAD) of misfolded proteins is activated, linking misfolded proteins accumulating in the lumen of the ER to ubiquitination and proteasome-dependent degradation. Several studies point indeed to a possible role of proteasome inhibition in the development of Alzheimer's disease (for a review see Checler *et al*. 2000).

A regulatory link between ERAD and UPR has been identified (Friedlander *et al*. 2000) which may be modulated in pathological processes associated with degenerative diseases. Parkin, the product of familial Parkinson's disease gene, has been shown to be a ubiquitin-protein ligase, and mutant parkins found in familial Parkison's disease patients

show loss of ubiquitin-protein ligase activity (Shimura *et al*. 2000). UPR induces an up-regulation of parkin, and cells overexpressing parkin, but not mutant parkins found in familial Parkinson's disease patients, are particularly resistant to unfolded protein-induced cell death (Imai *et al*. 2000). This suggests that neurons of patients suffering from familial Parkinson's disease are less well protected from neurotoxicity arising under conditions of ER stress.

Common denominators?

Accepting the notion that ER dysfunction is involved in the development of neuronal cell injury, processes may be identified which play a role in these pathological states of the brain and which may induce disturbances of ER function. One possible common denominator of processes culminating in neuronal cell injury in various pathological states of the brain is oxidative stress (for a recent review see Deschamps *et al*. 2001; Mattson *et al*. 2001). Oxidative stress induces a selective down-regulation of the neuronal UPR, and a close relationship has been observed between the extent of UPR suppression and the level of oxidative stress necessary to induce neuronal cell injury (Paschen *et al*. 2001). Furthermore, stroke or Alzheimer's disease and Parkinson's disease occur more frequently with age, suggesting that cellular senescence may lead to reduced tolerance of cells to conditions associated with ER stress. ER function is indeed deteriorating with age (see below). Another possible common denominator contributing to ER dysfunction in acute and chronic diseases of the brain is glutamate-induced excitotoxicity. As will be discussed below, ER dysfunction may contribute to glutamate-induced excitotoxicity, as after activation of a particular set of ionotropic glutamate receptor subtypes a large fraction of Ca^{2+} ions accumulating in the cytoplasm derives from ER stores implying a role of ER calcium store depletion in excitotoxin-induced neuronal cell injury.

Age

Assuming that ER dysfunction is a common global pathomechanism underlying neuronal cell injury in various pathologies, it would be interesting to establish whether ER function deteriorates with age and whether improving ER function has a beneficial effect on neurons. It has indeed been shown that neuronal ER calcium uptake, calcium levels and SERCA activity decline with age (Kirischuk and Verkhratsky 1996; Pottorf *et al*. 2000). A similar age-related change in ER calcium homeostasis has also been observed in non-neuronal cells such as fibroblasts (Huang *et al*. 2000), suggesting that disturbance of ER function is a common phenomenon concomitant to cellular senescence. Accepting that ER calcium homeostasis may not only be disturbed in neurons but also in non-neuronal cells, the age-related decline in ER calcium uptake may also be present in endothelial and smooth muscle cells of cerebral vessels, thus decreasing the calcium buffer capacity and increasing the risk of vessel constriction. This view is corroborated by the observation that functional elimination of the sarcoplasmic reticulum compartment causes marked contraction of cerebral arteries (Asano *et al*. 1996).

The assumption of a role of age-related ER dysfunction in cellular senescence is supported by results of experimental studies investigating the mechanisms underlying the effect of caloric restriction on incidence and severity of neurodegenerative disorders (for a review see Mattson 2000). In these experimental studies performed both *in vitro* and *in vivo*, caloric restriction was achieved by reducing the caloric intake or by administrating 2-deoxyglucose, a non-metabolizable analog of glucose (Lee *et al*. 1999; Yu *et al*. 1999; Guo and Mattson 2000). Caloric restriction offered neuronal protection against focal ischemia *in-vivo*, and against oxidative, excitotoxic and beta-amyloid-induced injury, and cell damage caused by apoptosis-inducing compounds (e.g. staurosporine) *in vitro*. This protection resulted from a suppression of oxidative stress and stabilization of calcium homeostasis. Evidence has been presented that such neuroprotection is caused by the rise in levels of the ER-resident protein GRP78 following caloric restriction: neuroprotection was completely suppressed when the usual rise in GRP78 protein levels after lowering of the caloric intake was blocked in cells treated with grp78 antisense DNA (Yu *et al*. 1999). Furthermore, grp78 antisense treated cells exhibited increased vulnerability towards oxidative stress and beta-amyloid toxicity (Yu *et al*. 1999), and the glutamate-induced rise in cytoplasmic calcium activity was markedly more pronounced in these cells (Yu *et al*. 1999). These observations suggest that a disturbance of ER calcium homeostasis may well contribute to glutamate excitotoxicity (see below).

Excitotoxicity

The excitotoxicity hypothesis holds that excessive activation of excitatory amino acid receptors contributes to the pathogenesis of neuronal cell injury in various pathological states of the brain, and that high cytoplasmic calcium levels induced by exposure to excitotoxins are the primary trigger for this process (for a review see Siesjö 1981). Exposure of neurons to the excitotoxic amino acid glutamate does indeed induce a marked increase in cytoplasmic calcium activity. However, after the activation of a particular set of glutamate receptor subtypes, a large portion of calcium ions present in the cytoplasm derives from ER calcium stores and it has been shown that both this increase in calcium activity and the development of cell injury can be blocked by dantrolene, an antagonist of the ER ryanodine receptor (Frandsen and Schousboe 1992). It has therefore been suggested that calcium influx may not necessarily be the primary cause of toxicity following exposure of neurons to the excitotoxins quisqualate, *N*-methyl-D-aspartate or glutamate (for a review

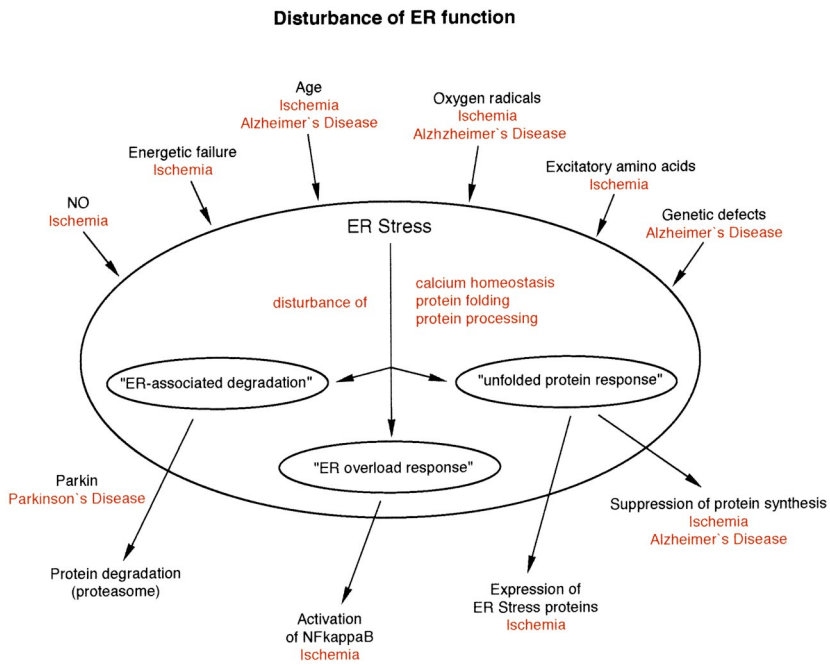

Fig. 1 Scheme of the possible mechanisms underlying ER dysfunction in acute pathological states of the brain (e.g. cerebral ischemia) and degenerative diseases. ER function may be disturbed with increasing age, under conditions associated with oxidative stress, energetic failure, or increased levels of NO or excitatory amino acids, and also in cells expressing mutant presenilins. Ischemia is the most commonly investigated acute pathological state of the brain but similar changes are induced by trauma, transient hypoglycemia or epileptic seizures.

see Frandsen and Schousboe 1993; Mody and Macdonald 1995). These observations are usually discussed in line with the traditional calcium hypothesis. However, as high ER calcium levels are necessary for the proper functioning of ER-resident processes such as protein folding and processing (see above), and as any major disturbance of these important ER-resident reactions will trigger programmed cell death (for a review see Kaufman 1999), the neuroprotective effect of dantrolene or any related compound blocking depletion of ER calcium stores could also arise from a stabilization of ER function.

The traditional calcium hypothesis has been modified to account for the observation that neuronal cell injury is induced under conditions associated with both increases and decreases in cytoplasmic calcium activity (Koike *et al*. 1989), and to allow for the fact that the extent of cell injury is not related to the increase in cytoplasmic calcium activity (Witt *et al*. 1994; Sattler *et al*. 1998; Sattler and Tymianski 2000). An alternative interpretation would be that it is ER calcium store depletion per se, rather than actual levels of cytoplasmic calcium activity that underlies the pathological process, as ER calcium stores are depleted both when cytoplasmic calcium activity is low (Doutheil *et al*. 1999) and under those experimental conditions where cell injury develops in the presence of high cytoplasmic calcium activity (exposure to *N*-methyl-D-aspartate or the calcium ionophore A23187). If ER dysfunction contributes directly to neuronal cell injury under conditions associated with depletion of ER calcium stores, we may also re-interpret pathogenetic mechanisms in experimental models of familial Alzheimer's disease, where mutant presenilins have been shown to lower the excitotoxic threshold (Chan *et al*. 2000; Schneider *et al*. 2001). In neuronal cells expressing these mutant presenilins, activation of ER calcium release induced an increase in cytoplasmic calcium activity compared with controls. These mutant presenilins excitotoxicity in the presence of caffeine, an agonist of the ryanodine receptor, was enhanced and prevented by dantrolene (Chan *et al*. 2000; Schneider *et al*. 2001). A larger increase in cytoplasmic calcium activity brought about by release of ER calcium was also observed in cells in which levels of the ER chaperone GRP78 had been reduced using the antisense technique (Yu *et al*. 1999). These are experimental conditions which are not believed to be associated with overfilled ER Ca^{2+} stores, suggesting that it is not necessarily the filling level of ER calcium store which is elevated but the amount of calcium ions released from these stores after receptor activation. Ryanodine receptor protein levels have indeed been shown to be markedly increased in cells expressing mutant presenilins (Chan *et al*. 2000), implying that excessive calcium release through the ryanodine receptor-activated calcium channel plays a critical role in enhancing excitotoxicity.

Conclusion

Evidence is presented that ER function is disturbed in acute and chronic diseases of the brain. These observations together with observations indicating that severe disturbances of ER function are sufficient to induce cell injury imply a direct cause relationship between ER dysfunction and induction of cell damage in these pathological states of the brain. The ER is the site of complex processes such as calcium storage and calcium signaling, processing and

folding of newly synthesized membrane and secretory proteins, and triggering of cell response to severe forms of stress, which in turn interfere with ER functions. Various different factors may induce ER dysfunction (Fig. 1), including age (decrease of ER calcium pump activity with age), metabolic stress, and genetic defects. Cell injury may develop under conditions where ER calcium homeostasis and/or folding or processing of proteins is disturbed, leading to the activation of the UPR, EOR and ERAD. Alternatively, the ability of cells to adapt to stress conditions associated with ER dysfunction may be impaired through a genetic defect such as a presenilin or parkin mutation, leading to down-regulation of the UPR. Knowledge of the exact mechanisms underlying ER dysfunction in different disorders of the brain will improve our chances of finding specific therapeutical strategies to impede the pathological processes directly.

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