Chapter 5
ER Stress

Masamitsu Shimazawa and Hideaki Hara

Abstract Glaucoma, an optic neuropathy resulting from retinal ganglion cell (RGC) death, is one of the leading causes of blindness worldwide. The causes of RGC death in glaucoma have been reported to arise from intraocular pressure, dysregulation of ocular circulation, autoimmune diseases, and genetic predisposition and so on. However, its pathological mechanisms remain unclear. Recently, it is focused on the involvement of endoplasmic reticulum (ER) stress in glaucoma. The authors demonstrated, for the first time, that various types of cellular stress induce ER stress before proceeding to RGC death. ER stress is caused by the accumulation of misfolded or unfolded proteins within the ER lumen. The excess ER stress leads to ER-stress-induced cell death, highlighting the possible mechanisms of neurodegenerative diseases, such as Alzheimer disease, amyotrophic lateral sclerosis, and Parkinson disease. This chapter introduces the involvement of ER stress in retinal cell death causing glaucoma and its therapeutic strategy.

Keywords Endoplasmic reticulum stress • Glucose-regulated protein 78 • Unfolded protein response

5.1 ER Stress and Neurodegeneration

In chronic neurodegenerative disorders such as Alzheimer disease, Parkinson disease, Huntington disease, and amyotrophic lateral sclerosis (ALS), abnormally unfolded proteins are known to aggregate and accumulate in neurons, and they are thought to be closely related to the initiation and development of these neurodegenerative diseases [1–3]. Recently, endoplasmic reticulum (ER) stress has been

M. Shimazawa (✉) • H. Hara
Molecular Pharmacology, Department of Biofunctional Evaluation, Gifu Pharmaceutical University, 1-5-25 Daigaku-nishi, Gifu 501-1196, Japan
e-mail: shimazawa@gifu-pu.ac.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_5, © Springer Japan 2014

reported to induce neuronal cell death and, moreover, to play roles in neurodegenerative diseases [3]. ER stress is caused by a number of biochemical and physiological stimuli that result in the accumulation of unfolded proteins in the ER lumen, and it is closely associated with the neuronal cell injury caused by vascular and neurodegenerative diseases such as stroke, Alzheimer disease, and Parkinson disease [1, 4, 5].

The ER is the cellular organelle in which secreted and transmembrane proteins are newly synthesized, posttranslationally modified, and properly folded to function. Agents or conditions that adversely affect ER protein folding lead to an accumulation of unfolded or misfolded proteins in the ER, a condition defined as ER stress. ER stress activates signaling pathways, including the unfolded protein response (UPR) that counteracts the effects of the original stress. The accumulation of unfolded or misfolded proteins in the ER causes ER stress; a complex signal-transduction cascade, known as UPR, is activated to cope with ER stress [2]. UPR is mediated by three types of ER transmembrane proteins: inositol-requiring enzyme 1 (IRE1), RNA-dependent protein kinase-like ER eukaryotic translation initiation factor 2α kinase (PERK), and activating transcription factor 6 (ATF6) [3], and the expression of both glucose-regulated protein 78 (GRP78)/BiP and C/EBP-homologous protein (CHOP) mRNAs is upregulated by the activation of these pathways (Fig. 5.1). UPR activates at least four pathways. One major component of the UPR is the elevated expression of molecular chaperones, such as GRP78/BiP, GRP94, and calreticulin, to increase protein folding activity and prevent protein aggregation [6]. Another component of UPR is the suppression of the protein burden through the global inhibition of translation. PERK is the sensor protein of UPR. The third component, ER-associated degradation, is extensive degradation of unfolded proteins. The final pathway induced by ER stress, CHOP, triggers cell cycle arrest and apoptosis [7]. ER stress can be induced by agents or conditions that interfere with (a) protein glycosylation (e.g., glucose starvation, tunicamycin, glucosamine), (b) disulfide-bond formation (e.g., DTT, homocysteine), (c) Ca^{2+} balance (A23187, thapsigargin, EGTA), and/or (d) a general overloading of the ER with proteins (e.g., viral or nonviral oncogenesis) [3, 6, 8].

5.1.1 ER Stress and Retinal Ganglion Cell Death

Retinal ganglion cell (RGC) death is a common feature of many ophthalmic disorders such as glaucoma, optic neuropathies, and retinovascular diseases, such as diabetic retinopathy and retinal vein occlusions. RGC death has been reported to occur via a variety of mechanisms involving, for example, oxidative stress [9], excitatory amino acids [10], nitric oxide (NO) [11], and apoptosis [12]. Glutamate, one of the excitatory amino acids, is the main neurotransmitter in the retinal signaling pathway. Excessive glutamate increases both intracellular Ca^{2+} and NO production through activation of the *N*-methyl-D-aspartate (NMDA)-type glutamate receptor, resulting in retinal cell death [13, 14]. However, little is known about the

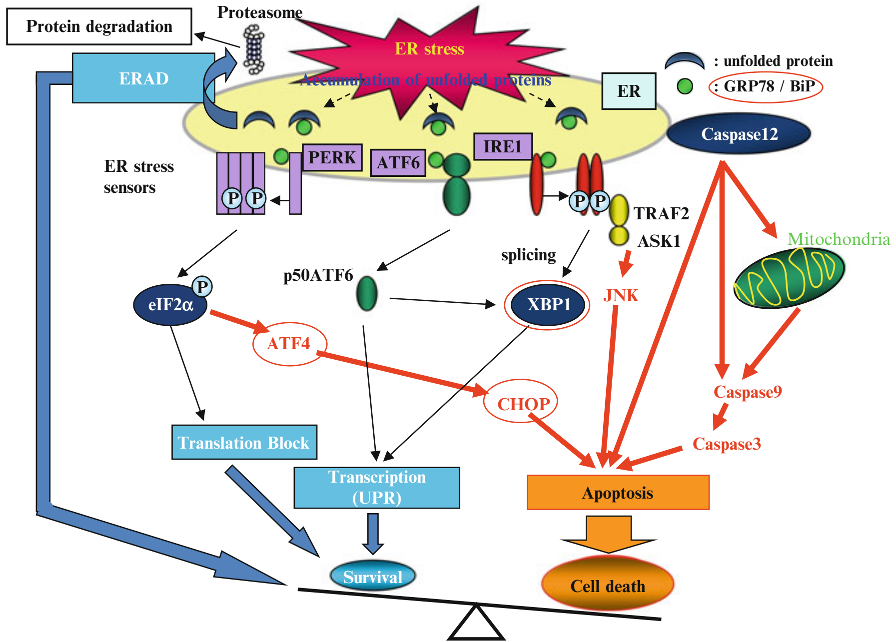

Fig. 5.1 ER-stress-signal pathways. *BiP*, glucose-regulated protein (GRP)78/BiP; *eIF2*α, eukaryotic initiation factor 2α; *CHOP*, C/EBP-homologous protein; *XBP-1*, X-box-binding protein 1; *ATF4*, activating transcription factor 4; *TRAF2*, TNF receptor-associated factor 2; *ASK1*, apoptosis signal-regulating kinase 1; *JNK*, c-Jun NH(2)-terminal kinase; *IRE1*, inositol-requiring enzyme 1; *PERK*, PKR-like ER kinase

role, if any, of ER stress in retinal damage. Recently, Uehara et al. [15] reported that in primary cortical culture, even mild exposure of NMDA induces apoptotic cell death. They demonstrated to be caused by an accumulation of polyubiquitinated proteins and increases in X-box-binding protein (XBP-1) mRNA splicing and CHOP mRNA, representing activation of the UPR signaling pathway. They also found that protein-disulfide isomerase (PDI), which assists in the maturation and transport of unfolded secretory proteins, prevented the neurotoxicity associated with ER stress. They suggest that neurodegenerative disorders might be mediated by S-nitrosylation of PDI, which would reduce its enzymatic activity. Their results strongly suggest that the activation of ER stress may participate in the retinal cell death occurring after NMDA-receptor activation and/or ischemic insult.

NMDA receptors may participate in the processes of excitotoxicity and neuronal death in the retina [16, 17]. Previous studies have found that TUNEL-positive cells can be observed in the retinal ganglion cell layer (GCL) and inner nuclear layer (INL) of the mouse retina at an early stage (within 24 h) after an intravitreal injection of NMDA [18, 19]. The hallmark of NMDA-induced neuronal death is a sustained increase in the intracellular Ca^{2+} concentration accompanied by overactivation of vital Ca^{2+}-dependent cellular enzymes [20]. Thus, the signal-transduction

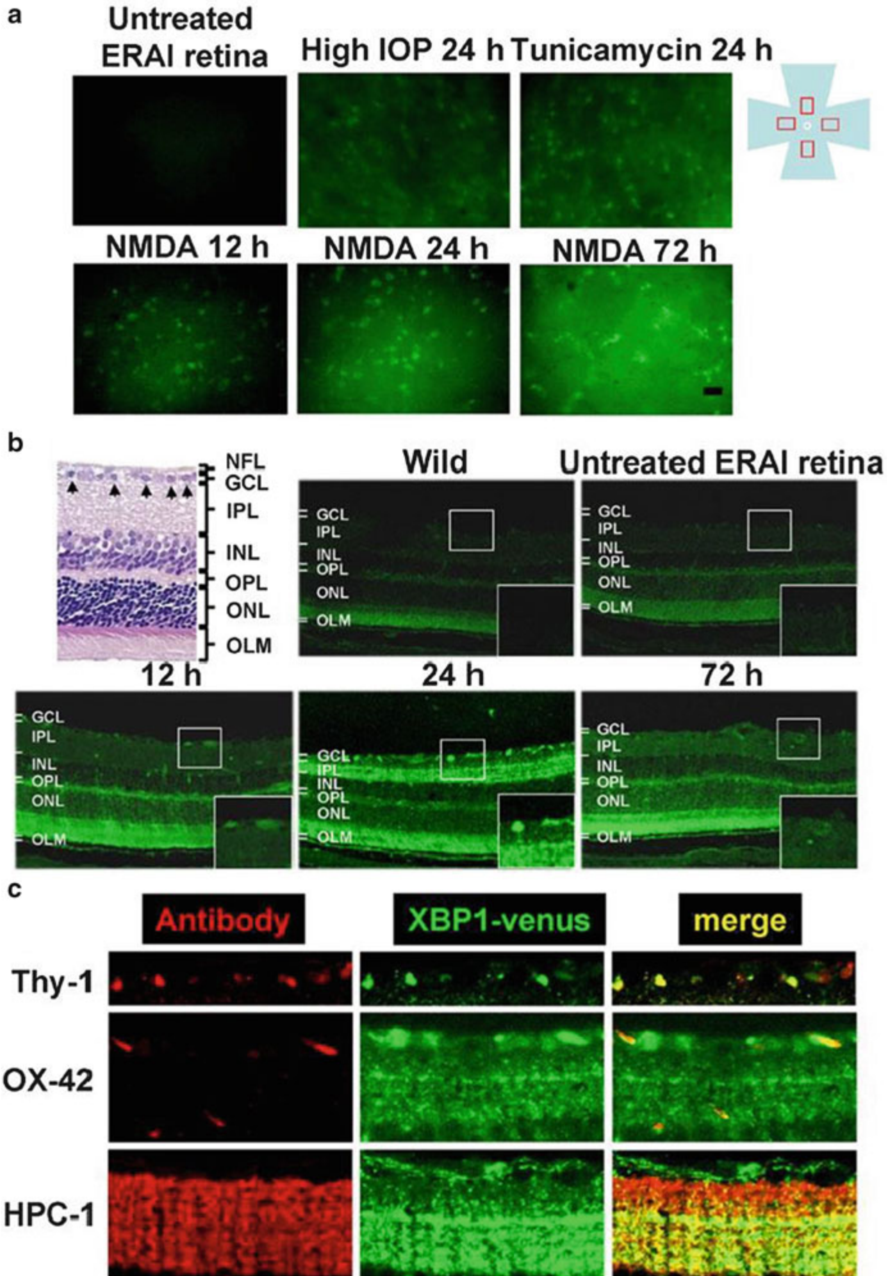

Fig. 5.2 Expression and localization of XBP-1–venus fusion protein in ERAI mouse retinas after various types of retinal damage. (**a**) Representative fluorescence photographs of increased XBP-1–venus fusion protein in ERAI mouse flat-mounted retina after *N*-methyl-D-aspartate (NMDA), intraocular pressure (IOP) elevation, or tunicamycin insult. The fluorescence (*green*) arising from

pathways for NMDA-mediated cell death in the retina are well studied, but not yet fully understood. To illuminate the role and distribution of ER stress in vivo, we focused on the retina of ER-stress-activated indicator (ERAI) transgenic mice carrying a human XBP-1 and venus, a variant of green fluorescent protein (GFP) fusion gene, in which effective identification of cells under ER-stress conditions is possible in vivo, as described in our previous report [21]. In flat-mounted retinas, fluorescence arising from the XBP-1–venus fusion protein was detected following various stimulations [tunicamycin, NMDA, and intraocular pressure (IOP) elevation] (Fig. 5.2). To our knowledge, this is the first report demonstrating that NMDA and ischemic insult (elevating IOP), in addition to tunicamycin, can activate the ER-stress signal (measured as the splicing of the XBP-1 and venus fusion gene in ERAI transgenic mice) in the retina in vivo. Interestingly, ER stress was also induced in the retina after a transient IOP elevation, defined as an ischemia–reperfusion model. It has been reported that this model exhibits retinal cell damage similar to that induced by NMDA and that both of these examples of damage are protected against by dizocilpine, an NMDA-receptor antagonist, and by NO synthetase-inhibitor treatment [13, 22]. Although little is known about the precise mechanisms responsible for activation of ER stress after NMDA or IOP elevation (ischemia–reperfusion), both stimuli cause intracellular Ca^{2+} overload and increased NO production, resulting in apoptotic cell death. Several lines of study suggest that intracellular Ca^{2+} overload and excessive production of NO deplete Ca^{2+} in the ER, thereby resulting in ER stress [23, 24]. Uehara et al. [15] reported that NO induces S-nitrosylation of PDI, an enzyme that assists in the maturation and transport of unfolded secretory proteins and thereby helps to prevent the neurotoxicity associated with ER stress. S-nitrosylated PDI exhibits reduced enzymatic activity and induces cell death through the ER-stress pathway. These mechanisms may contribute to the activation of ER stress in the retina after NMDA stimulation or IOP elevation. Accordingly, our findings may provide important new insights into the mechanisms underlying the retinal cell damage induced by NMDA and by ischemia–reperfusion. In transverse retinal sections, we observed an increase in fluorescence intensity within the cells of the GCL and IPL at 12 and 24 h, respectively, after NMDA injection. The cells displaying increased fluorescence were ganglion cells (at 12 h after the injection), amacrine cells in IPL (at 24 h), and

Fig. 5.2 (continued) XBP-1–venus fusion protein was observed under an epifluorescence microscope. The scale bar represents 25 μm. (**b**) Distribution of increased XBP-1–venus fusion protein in retinal cross sections from ERAI mice after NMDA injection at 40 nmol/eye. The distribution of fluorescence (*green*) arising from XBP-1–venus fusion protein was observed under a laser confocal microscope. Each large box shows an enlargement of the area within the corresponding small box. (**c**) Localization of XBP-1–venus fusion protein in ERAI mouse retina after NMDA injection. In the retinal nerve fiber layer (*upper panels*), Thy-1-positive cells (*red*) can be seen to merge with XBP-1–venus fusion protein (*green*). In the *middle panels*, OX-42 (a microglia marker)-positive cells (*red*) are partly merged with XBP-1–venus fusion protein (*green*). In the inner plexiform layer (*lower panels*), HPC-1 (an amacrine marker)-positive cells (*red*) are merged with XBP-1–venus fusion protein (*green*). Modified from Shimazawa et al. [95]

microglia in GCL (at 72 h). These data indicate that ganglion cells may be more sensitive to ER stress than the other retinal cells examined. To further clarify the participation of ER stress, we examined the changes in GRP78/BiP and CHOP in the retina after NMDA-induced injury. We found (a) that NMDA induced GRP78/BiP proteins in the retina at 12 h after its injection (on the basis of immunoblots) and (b) that NMDA induced both GRP78/BiP and CHOP in the retina (especially within retinal ganglion cells and INL) at 12 h after its injection (on the basis of our immunostaining results). The expression of the CHOP gene reportedly increases in the rat retina after intravitreal injection of NMDA [25]. Furthermore, Awai et al. [26] found that treatment with MK-801, an NMDA-receptor antagonist, inhibited the increases in CHOP mRNA and protein in the mouse retina that are observed after intravitreal injection of NMDA and moreover that CHOP-deficient mice were resistant to NMDA-induced retinal damage. However, CHOP-deficient mice partially suppressed the NMDA-induced cell death, and therefore other pathways, such as mitochondrial dysfunction, may be engaged in the retinal cell death. Collectively, the above results indicate that NMDA can cause ER stress in the retina and that the neurotoxicity induced by NMDA is due in part to a mechanism dependent on CHOP protein induction through excessive ER stress. In conclusion, we have identified a close association between ER stress and retinal damage, and these results suggest that the ER-stress-signal pathway might be a good target in the treatment of retinal diseases.

5.1.2 ER Stress in Glaucoma

Glaucoma is a multifactorial optic neuropathy characterized by RGC death [27]. This irreversible RGC death results in progressive visual field loss along with decreased color sensitivity and contrast [28]. Although RGC death can be observed in patients with normal ocular tension [29], if genetic, environmental, and other factors are involved [30], elevated IOP is a recognized risk factor for RGC degeneration in glaucoma. At present, the only well-established treatment of glaucoma involves lowering the IOP; however, visual field loss continues to progress in a subset of glaucoma patients even if medical and surgical treatments successfully lower the IOP [31]. Thus, new approaches to treating glaucoma, such as directly preventing RGC death, have been required in addition to regulating IOP, but the associated pathological mechanisms remain unclear. Therefore, further studies will be needed to clarify the precise mechanisms in glaucoma pathogenesis.

Previous studies suggest that there is a significantly higher rate of glaucoma occurrence among patients with Alzheimer disease (AD), the most common form of dementia, than control subjects, suggesting a possible relationship between these two diseases [32]. Briefly, Bayer et al. [32] noted that Alzheimer's patients had a greater rate of glaucoma occurrence (25.9 %) than a control group (5.2 %). Subsequently, we reported (a) that the concentrations of $A\beta_{1-42}$ and tau were decreased and increased, respectively, in vitreous fluid from patients with glaucoma and

diabetic retinopathy (vs. macular hole controls) [33], (b) that in AD transgenic mice (Tg2576/PS1 mutant), there was a significant decrease in the visual function [34], and (c) that β-secretase inhibitors reduced glutamate-induced cell death in rat primary cultured retinal ganglion cells [35]. On the other hand, a chronic elevation of IOP induces Aβ in RGCs in experimental rat glaucoma [30]. This result is consistent with some reports on experimental glaucoma models of rats [36] and mice [37]. Furthermore, Guo et al. [36] reported that neutralizing antibody to Aβ significantly delays and attenuates RGC apoptosis in experimental glaucoma. These findings indicate that $A\beta_{1-42}$ neurotoxicity as AD brain may be involved in RGC death in glaucoma; however, the study by Guo and colleagues [36] did not use primate, but rodents (mouse and rat) for the study of experimental glaucoma. Recently, we found that the expression of $A\beta_{1-42}$ was increased in the retina and the optic nerve head (ONH) of monkeys with experimental glaucoma [38]. Thus, accumulations of Aβ in the retina and ONH may be involved in glaucoma pathogenesis. So far, many researchers [39–42] reported that the accumulation of Aβ may be associated with ER stress in the brains of AD model mice and AD patients; therefore, ER stress may be closely related to RGC death in glaucoma. Doh et al. [43] investigated whether ER stress induced RGC death in chronic ocular hypertension, one of the RGC death mechanisms, using an experimental glaucoma rat model and demonstrated that GRP78/BiP, p-PERK, and CHOP were significantly expressed in the retina with chronic IOP elevation. This finding strongly suggests that ER stress is involved in RGC death in glaucoma, and the PERK–p-eIF2α–CHOP pathway plays a role in the RGC death associated with ER stress. Recently, Yang et al. [44] reported that the expression of various ER-resident proteins, including GRP78/BiP and PDI, and ER sensor proteins detecting UPR including ATF6 and IRE1 was increased in the retina of glaucoma patients. These findings strongly supported the involvement of ER stress in glaucoma pathogenesis, but further studies will be needed to demonstrate it.

5.1.3 ER Stress in Lateral Geniculate Nucleus of Glaucoma

Recent evidence indicates that glaucomatous damage extends from the retina to the visual center of the brain, including the lateral geniculate nucleus (LGN) and the primary visual cortex [45–47]. In particular, neuronal damage in the LGN in monkey glaucoma models can be detected in the early phase (the first few weeks) after IOP elevation [48–50]. Furthermore, relay neurons, a type of LGN neuron that proceed to synapse in the visual cortex, were more vulnerable than the other types of LGN neurons after IOP elevation [51, 52]. In addition, most of the degenerative and compensatory changes in the LGN occur in the relay neurons after total deafferentation [53, 54]. Therefore, protecting relay and retinal neurons may be effective in preventing blindness in glaucoma cases because visual information entering the eye is processed in the retina and then transmitted to the LGN, from where signals are relayed to the visual cortex.

In the LGN, the relay neurons predominately express the NMDA receptor [55–58]. In glaucoma models, excessive activation of this receptor leads to an overload of intracellular Ca^{2+} [59, 60]. Such elevations in Ca^{2+} elicit the activation of NO formation through NO-synthase activation [15, 61–63]. NO induces S-nitrosylation of PDI and inhibits its enzymatic activity as described in Sect. 5.1.1 [15]. As a consequence, this leads to excess accumulation of misfolded or unfolded proteins within the ER that may lead to ER-stress-induced cell death via the p-eIF2α–CHOP signal pathway [15, 64–66]. The involvement of ER stress in relay neuronal atrophy and death within the LGN via the NMDA–Ca^{2+}–NO synthase/NO pathway after IOP elevation supports our results: memantine, an NMDA antagonist, and lomerizine, a Ca^{2+} channel blocker, reduced neuronal atrophy in the LGN after retinal damage [60, 67]. Furthermore, we demonstrated that TUNEL-positive apoptotic cells and the production of ER-stress-related proteins were increased in LGN neurons of glaucoma monkeys [68]. Regarding the localization of ER-stress-related proteins, parvalbumin-positive relay neurons in the LGN layers connected to the eye with elevated IOP were found to express p-eIF2a and CHOP at 11–24 weeks after chronic IOP elevation. Delayed neuronal death in these regions progressed gradually following RGC axon loss by IOP elevation. As expression of the ER-stress-related proteins measured here may be detected before dying cells at each sampling point (i.e., at 4, 11, 15, and 24 weeks after IOP elevation), the number of cells positive for ER-stress markers may be relatively low in comparison with the number (and extent) of parvalbumin-positive relay neuron loss in the LGN. The total number of detectable neuron loss in the LGN, but not cells positive for ER-stress markers, increased during the study period. Therefore, the number of ER-stress-positive cells in the LGN at each sampling point may be smaller than those undergoing neuronal cell death. In this context, it is noteworthy that the timing of the upregulation of p-eIF2α and CHOP proteins coincided with the timing of the decrease in neuronal cells during the study period and these ER-stress-related proteins were co-localized with parvalbumin-positive relay neurons. Furthermore, the increase in polyubiquitinated proteins was preceded by an increase in TUNEL-positive cells in the LGN after the laser photocoagulation treatment during the study period. These findings indicate that excessive ER stress (caused by the accumulation of misfolded or unfolded proteins) induces LGN neuronal death via the activation of the ER-dependent apoptotic pathway, suggesting that the ER-stress pathway may play an important role in LGN neuronal death after IOP elevation. On the other hand, an increase in GRP78/BiP was not detected in the LGN after IOP elevation during the study period as assessed by immunostaining (data not shown). A possible explanation for this observation is a direct cytotoxic effect of p-eIF2α [69] and activation of the caspase-3 pathway [70–72], whereby p-eIF2α directly mediates apoptosis in response to activation of the double-stranded RNA-dependent protein kinase (PKR). In fact, we have previously shown that inhibition of PKR activation was neuroprotective against ER-stress-induced RGC death [73]. Although GRP78/BiP expression requires further studies, the present data suggest that impaired induction of antiapoptotic GRP78/BiP is accompanied by a strong induction of proapoptotic signal in the ER, indicating a signal imbalance

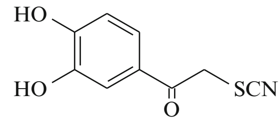

Fig. 5.3 Chemical structure of 1-(3,4-dihydroxyphenyl)-2-thiocyanate-ethanone (BIX)

leaning toward cell death. We have previously shown that a preferential inducer of GRP78/BiP exhibited the potential to be a therapeutic agent for ER-stress-induced retinal diseases [74–76]. In conclusion, the present study indicates that ER stress may be involved in LGN neuronal death after IOP elevation and the upregulation of p-eIF2α and CHOP protein levels in the parvalbumin-positive relay neurons may play roles in the cell death process induced by high IOP in monkey. These findings also indicate that ER stress induced by retinal damage may play a pivotal role in the pathogenesis of the blindness caused by retinal diseases such as glaucoma.

5.1.4 ER Stress Targeting Agents for Neuroprotection in Glaucoma

GRP78/BiP, a highly conserved member of the 70 kDa heat-shock protein family, is one of the chaperones localized to the ER membrane [23, 77], and it is a major ER-luminal Ca^{2+}-storage protein [78, 79]. GRP78/BiP works to restore folding in misfolded or incompletely assembled proteins [80–82], the interaction between BiP and misfolded proteins being dependent on its hydrophobic motifs [83–85]. Proteins stably bound to BiP are subsequently translocated from the ER into the cytosol, where they are degraded by proteasomes [86, 87]. Previous reports have show that induction of BiP prevents the neuronal death induced by ER stress [88–91]. Hence, a selective inducer of BiP might attenuate ER stress and be a new, useful therapeutic agent for the treatment of ER-stress-associated diseases. This seemed an interesting idea, and we recently identified BiP inducer X (BIX, Fig. 5.3) while screening for low-molecular-mass compounds that might induce BiP using high-throughput screening with a BiP reporter assay system (Dual-Luciferase Reporter Assay; Promega Corporation, Madison, WI) [74]. We found that BIX preferentially induced BiP mRNA and protein in SK-N-SH cells and reduced tunicamycin-induced cell death. Intracerebroventricular pretreatment with BIX reduced the infarction size after focal cerebral ischemia in mice. In view of the retinal research described above, we wondered whether BIX might reduce the retinal ganglion cell loss and CHOP expression induced by tunicamycin or NMDA treatment.

BIX preferentially induced GRP78/BiP mRNA in RGC-5 (a retinal precursor cell line) [76]. Although it also induced GRP94, calreticulin, p58IPK, and ASNS, these inductions were lower than that of GRP78/BiP. This is consistent with our previous study that BIX preferentially induced BiP with slight inductions of GRP94, calreticulin, and CHOP mediated by the ATF6 pathway accompanied by

activation of ERSEs and that BIX does not affect the pathway downstream of IRE1 or the translational control branch downstream of PERK in SK-N-SH cells [74]. Therefore, BIX is not just an ER stressor such as tunicamycin or thapsigargin, and we consider that the induction of GRP78/BiP by BIX is mediated by the ATF6 pathway in RGC-5 similar to that in SK-N-SH cells. Next, we evaluated the effects of BIX, as a preferential inducer of GRP78/BiP, on ER-stress-induced in vitro cell death in RGC-5 and in vivo retinal damage in mice. BIX reduced tunicamycin-induced cell death in RGC-5 and also reduced both tunicamycin-induced and NMDA-induced retinal damage in mice [76]. Our previous study revealed that BIX (a) reduced tunicamycin-induced cell death in SK-N-SH cells, (b) contributed to the induction of GRP78/BiP expression via the ATF-6 pathway (but not via the PERK or IRE1 pathways), and (c), on intracerebroventricular injection, prevented the neuronal damage induced by focal ischemia in mice [74]. Furthermore, immunostaining revealed that intravitreal injection of BIX significantly induced GRP78/BiP protein in mouse retina. On the other hand, there was little protective effect of BIX against RGC-5 damages after staurosporine treatment. Staurosporine is well known as a nonspecific inhibitor of protein kinases and initiates caspase-dependent apoptosis in many cell types [92, 93]. Our previous studies revealed that staurosporine induced cell death without any changes in the expression of BiP or CHOP protein [73, 94]. Furthermore, a preliminary study showed that treatment with BIX (1 and 5 μM) did not inhibit RGC-5 cell death 48 h after serum deprivation, which does not induce any UPR responses such as BiP or CHOP (unpublished data). These results strongly support that BIX selectively protects cell damage induced by ER stress. Recently, we reported that in mice, increased expressions of XBP-1 splicing, BiP, and CHOP could be detected after the induction of retinal damage by tunicamycin, NMDA, or an elevation of IOP [95]. That report was the first to demonstrate an involvement of ER stress and BiP in retinal cell death in mice. Hence, we asked whether BIX can prevent such retinal damage. By histologic analysis and TUNEL staining, we estimated that BIX reduced tunicamycin-induced retinal damage. Furthermore, we used Thy-1–CFP transgenic mice to examine the effect of BIX in a large retinal area [96]. This transgene contains a CFP gene under the direction of regulatory elements derived from the mouse Thy-1 gene, and the transgenic mice express CFP protein in RGC and in the inner part of the IPL of the retina [96]. BIX exerted protective effects against tunicamycin-induced retinal damage in the Thy-1–CFP transgenic mice (Fig. 5.4) and NMDA-induced retinal damage in ddY mice. NMDA is well known to induce RGC death and optic nerve loss (effects mediated by excitatory glutamate receptor), and such neuronal death is believed to play a role in many neurologic and neurodegenerative diseases [14, 97]. Uehara et al. [15] noted that mild exposure to NMDA induced apoptotic cell death in primary cortical culture, and they demonstrated this effect to be caused by an accumulation of polyubiquitinated proteins and increases in XBP-1 mRNA splicing and CHOP mRNA (reflecting activation of the UPR signaling pathway). They also found that PDI, which assists in the maturation and transport of unfolded secretory proteins, prevented the neurotoxicity associated with ER stress. These findings suggested that activation of ER stress

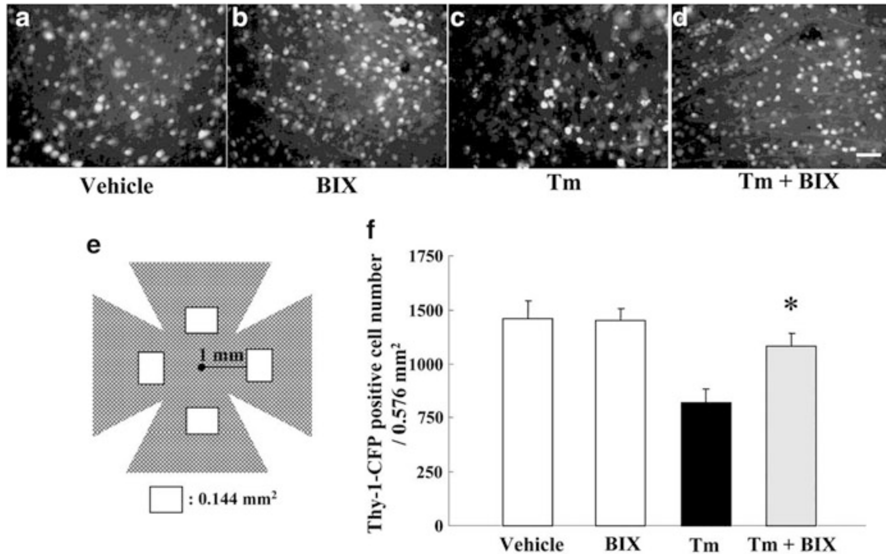

Fig. 5.4 Effects of BIX on retinal damage induced by intravitreal injection of tunicamycin (Tm) in Thy-1–CFP transgenic mice. Mouse retinas (flat mounts) at 7 days after intravitreal injection of (**a**) vehicle, (**b**) BIX (5 nmol), (**c**) Tm (1 μg), or (**d**) Tm (1 μg) plus BIX (5 nmol). Damage was evaluated by counting Thy-1–CFP-positive cell numbers in the four *white areas* shown in (**e**) (each area 0.144 $mm^2 \times 4$ areas; total 0.576 mm^2) at 7 days after the above intravitreal injections. (**f**) Effect of BIX against Tm-induced damage (indicated by decreased number of Thy-1–CFP-positive cells) at 7 days after intravitreal injection. Data are shown as mean ± SE ($n = 9$ or 10). $*P < 0.05$ versus tunicamycin alone. Scale bar represents 25 μm. Modified from Inokuchi et al. [76]

may participate in the retinal cell death occurring after NMDA-receptor activation and/or an ischemic insult [15]. BIX also attenuated the CHOP protein expression induced by either tunicamycin or NMDA in the mouse retina in vivo. As mentioned above, BIX may affect CHOP protein expression through ATF6 pathway, but no change was observed in BIX-treated RGC-5. In SK-N-SH cells, BIX slightly increased CHOP mRNA only at 2 h after the treatment. Expression of CHOP is mainly regulated by three transcription factors—ATF4, cleaved ATF6, and XBP-1—which are downstream effectors during ER stress in similar to other ER chaperones. These differences between BiP and CHOP expression by BIX may be due to the difference of their promoters. CHOP promoter contains at least two ERSE motifs (CHOP ERSE-1 and CHOP ERSE-2) located in opposite directions with a 9 bp overlap, and one of ERSEs is inactive [98]. On the other hand, BiP promoter has three functional ERSE motifs of the rat GRP78/BiP promoter (ERSE-163, ERSE-131, and ERSE-98) [99]. These variations in each promoter may contribute to the differences among the expressions of ER chaperons induced by BIX and the lack of CHOP expression.

In conclusion, we have demonstrated that BIX, a preferential inducer of BiP, inhibits both the neuronal cell death induced by ER stress in vitro in RGC-5 cells and in vivo in the mouse retina. Hence, an increase in BiP might be one of the targets of mechanisms bestowing neuroprotection in retinal diseases.

References

1. Aridor M, Balch WE (1999) Integration of endoplasmic reticulum signaling in health and disease. Nat Med 5(7):745–751
2. Harding HP, Calfon M, Urano F, Novoa I, Ron D (2002) Transcriptional and translational control in the Mammalian unfolded protein response. Annu Rev Cell Dev Biol 18:575–599
3. Kaufman RJ (1999) Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls. Genes Dev 13(10):1211–1233
4. Bando Y, Onuki R, Katayama T, Manabe T, Kudo T, Taira K, Tohyama M (2005) Double-strand RNA dependent protein kinase (PKR) is involved in the extrastriatal degeneration in Parkinson's disease and Huntington's disease. Neurochem Int 46(1):11–18
5. Gale M Jr, Katze MG (1998) Molecular mechanisms of interferon resistance mediated by viral-directed inhibition of PKR, the interferon-induced protein kinase. Pharmacol Ther 78 (1):29–46
6. Lee AS (2001) The glucose-regulated proteins: stress induction and clinical applications. Trends Biochem Sci 26(8):504–510
7. Oyadomari S, Mori M (2004) Roles of CHOP/GADD153 in endoplasmic reticulum stress. Cell Death Differ 11(4):381–389
8. Pahl HL (1999) Signal transduction from the endoplasmic reticulum to the cell nucleus. Physiol Rev 79(3):683–701
9. Bonne C, Muller A, Villain M (1998) Free radicals in retinal ischemia. Gen Pharmacol 30 (3):275–280
10. Dreyer EB (1998) A proposed role for excitotoxicity in glaucoma. J Glaucoma 7(1):62–67
11. Neufeld AH (1999) Nitric oxide: a potential mediator of retinal ganglion cell damage in glaucoma. Surv Ophthalmol 43(Suppl 1):S129–S135
12. McKinnon SJ (1997) Glaucoma, apoptosis, and neuroprotection. Curr Opin Ophthalmol 8 (2):28–37
13. Adachi K, Kashii S, Masai H, Ueda M, Morizane C, Kaneda K, Kume T, Akaike A, Honda Y (1998) Mechanism of the pathogenesis of glutamate neurotoxicity in retinal ischemia. Graefes Arch Clin Exp Ophthalmol 236(10):766–774
14. Sucher NJ, Lipton SA, Dreyer EB (1997) Molecular basis of glutamate toxicity in retinal ganglion cells. Vision Res 37(24):3483–3493. doi:10.1016/S0042-6989(97)00047-3
15. Uehara T, Nakamura T, Yao D, Shi ZQ, Gu Z, Ma Y, Masliah E, Nomura Y, Lipton SA (2006) S-nitrosylated protein-disulphide isomerase links protein misfolding to neurodegeneration. Nature 441(7092):513–517
16. Sabel BA, Sautter J, Stoehr T, Siliprandi R (1995) A behavioral model of excitotoxicity: retinal degeneration, loss of vision, and subsequent recovery after intraocular NMDA administration in adult rats. Exp Brain Res 106(1):93–105
17. Siliprandi R, Canella R, Carmignoto G, Schiavo N, Zanellato A, Zanoni R, Vantini G (1992) N-methyl-D-aspartate-induced neurotoxicity in the adult rat retina. Vis Neurosci 8(6):567–573
18. Hara A, Niwa M, Kumada M, Kitaori N, Yamamoto T, Kozawa O, Mori H (2004) Fragmented DNA transport in dendrites of retinal neurons during apoptotic cell death. Brain Res 1007(1–2):183–187
19. Li Y, Schlamp CL, Nickells RW (1999) Experimental induction of retinal ganglion cell death in adult mice. Invest Ophthalmol Vis Sci 40(5):1004–1008

20. Fukunaga K, Soderling TR, Miyamoto E (1992) Activation of Ca^{2+}/calmodulin-dependent protein kinase II and protein kinase C by glutamate in cultured rat hippocampal neurons. J Biol Chem 267(31):22527–22533
21. Iwawaki T, Akai R, Kohno K, Miura M (2004) A transgenic mouse model for monitoring endoplasmic reticulum stress. Nat Med 10(1):98–102
22. Lam TT, Siew E, Chu R, Tso MO (1997) Ameliorative effect of MK-801 on retinal ischemia. J Ocul Pharmacol Ther 13(2):129–137
23. Li WW, Alexandre S, Cao X, Lee AS (1993) Transactivation of the grp78 promoter by Ca^{2+} depletion. A comparative analysis with A23187 and the endoplasmic reticulum Ca(2+)-ATPase inhibitor thapsigargin. J Biol Chem 268(16):12003–12009
24. Oyadomari S, Araki E, Mori M (2002) Endoplasmic reticulum stress-mediated apoptosis in pancreatic beta-cells. Apoptosis 7(4):335–345
25. Laabich A, Li G, Cooper NG (2001) Characterization of apoptosis-genes associated with NMDA mediated cell death in the adult rat retina. Brain Res Mol Brain Res 91(1–2):34–42
26. Awai M, Koga T, Inomata Y, Oyadomari S, Gotoh T, Mori M, Tanihara H (2006) NMDA-induced retinal injury is mediated by an endoplasmic reticulum stress-related protein, CHOP/GADD153. J Neurochem 96(1):43–52
27. Quigley HA, Broman AT (2006) The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 90(3):262–267. doi:10.1136/bjo.2005.081224
28. Mozaffarieh M, Grieshaber MC, Flammer J (2008) Oxygen and blood flow: players in the pathogenesis of glaucoma. Mol Vis 14:224–233
29. Tomita G (2000) The optic nerve head in normal-tension glaucoma. Curr Opin Ophthalmol 11 (2):116–120
30. McKinnon SJ, Lehman DM, Kerrigan-Baumrind LA, Merges CA, Pease ME, Kerrigan DF, Ransom NL, Tahzib NG, Reitsamer HA, Levkovitch-Verbin H, Quigley HA, Zack DJ (2002) Caspase activation and amyloid precursor protein cleavage in rat ocular hypertension. Invest Ophthalmol Vis Sci 43(4):1077–1087
31. The effectiveness of intraocular pressure reduction in the treatment of normal-tension glaucoma. Collaborative normal-tension glaucoma study group (1998) Am J Ophthalmol 126 (4):498–505. doi:S0002939498002724
32. Bayer AU, Ferrari F, Erb C (2002) High occurrence rate of glaucoma among patients with Alzheimer's disease. Eur Neurol 47(3):165–168
33. Yoneda S, Hara H, Hirata A, Fukushima M, Inomata Y, Tanihara H (2005) Vitreous fluid levels of beta-amyloid((1-42)) and tau in patients with retinal diseases. Jpn J Ophthalmol 49 (2):106–108
34. Shimazawa M, Inokuchi Y, Okuno T, Nakajima Y, Sakaguchi G, Kato A, Oku H, Sugiyama T, Kudo T, Ikeda T, Takeda M, Hara H (2008) Reduced retinal function in amyloid precursor protein-over-expressing transgenic mice via attenuating glutamate-N-methyl-d-aspartate receptor signaling. J Neurochem 107(1):279–290. doi:10.1111/j.1471-4159.2008.05606.x, JNC5606 [pii]
35. Yamamoto R, Yoneda S, Hara H (2004) Neuroprotective effects of beta-secretase inhibitors against rat retinal ganglion cell death. Neurosci Lett 370(1):61–64
36. Guo L, Salt TE, Luong V, Wood N, Cheung W, Maass A, Ferrari G, Russo-Marie F, Sillito AM, Cheetham ME, Moss SE, Fitzke FW, Cordeiro MF (2007) Targeting amyloid-beta in glaucoma treatment. Proc Natl Acad Sci U S A 104(33):13444–13449. doi:10.1073/pnas.0703707104, 0703707104 [pii]
37. Goldblum D, Kipfer-Kauer A, Sarra GM, Wolf S, Frueh BE (2007) Distribution of amyloid precursor protein and amyloid-beta immunoreactivity in DBA/2J glaucomatous mouse retinas. Invest Ophthalmol Vis Sci 48(11):5085–5090. doi:10.1167/iovs.06-1249
38. Ito Y, Shimazawa M, Tsuruma K, Mayama C, Ishii K, Onoe H, Aihara M, Araie M, Hara H (2012) Induction of amyloid-beta(1-42) in the retina and optic nerve head of chronic ocular hypertensive monkeys. Mol Vis 18:2647–2657

39. Soejima N, Ohyagi Y, Nakamura N, Himeno E, Iinuma KM, Sakae N, Yamasaki R, Tabira T, Murakami K, Irie K, Kinoshita N, LaFerla FM, Kiyohara Y, Iwaki T, Kira J (2013) Intracellular accumulation of toxic turn amyloid-beta is associated with endoplasmic reticulum stress in Alzheimer's disease. Curr Alzheimer Res 10(1):11–20
40. Lee JH, Won SM, Suh J, Son SJ, Moon GJ, Park UJ, Gwag BJ (2010) Induction of the unfolded protein response and cell death pathway in Alzheimer's disease, but not in aged Tg2576 mice. Exp Mol Med 42(5):386–394. doi:10.3858/emm.2010.42.5.040
41. Hosoi T, Ozawa K (2012) Molecular approaches to the treatment, prophylaxis, and diagnosis of Alzheimer's disease: endoplasmic reticulum stress and immunological stress in pathogenesis of Alzheimer's disease. J Pharmacol Sci 118(3):319–324
42. Scheper W, Nijholt DA, Hoozemans JJ (2011) The unfolded protein response and proteostasis in Alzheimer disease: preferential activation of autophagy by endoplasmic reticulum stress. Autophagy 7(8):910–911
43. Doh SH, Kim JH, Lee KM, Park HY, Park CK (2010) Retinal ganglion cell death induced by endoplasmic reticulum stress in a chronic glaucoma model. Brain Res 1308:158–166. doi:10.1016/j.brainres.2009.10.025
44. Yang X, Luo C, Cai J, Powell DW, Yu D, Kuehn MH, Tezel G (2011) Neurodegenerative and inflammatory pathway components linked to TNF-alpha/TNFR1 signaling in the glaucomatous human retina. Invest Ophthalmol Vis Sci 52(11):8442–8454. doi:10.1167/iovs.11-8152
45. Gupta N, Yucel YH (2003) Brain changes in glaucoma. Eur J Ophthalmol 13(Suppl 3):S32–S35
46. Vrabec JP, Levin LA (2007) The neurobiology of cell death in glaucoma. Eye (Lond) 21(Suppl 1):S11–S14. doi:10.1038/sj.eye.6702880
47. Yucel Y, Gupta N (2008) Glaucoma of the brain: a disease model for the study of transsynaptic neural degeneration. Prog Brain Res 173:465–478. doi:10.1016/S0079-6123(08)01132-1
48. Weber AJ, Chen H, Hubbard WC, Kaufman PL (2000) Experimental glaucoma and cell size, density, and number in the primate lateral geniculate nucleus. Invest Ophthalmol Vis Sci 41(6):1370–1379
49. Ito Y, Shimazawa M, Chen YN, Tsuruma K, Yamashima T, Araie M, Hara H (2009) Morphological changes in the visual pathway induced by experimental glaucoma in Japanese monkeys. Exp Eye Res 89(2):246–255. doi:10.1016/j.exer.2009.03.013
50. Shimazawa M, Ito Y, Inokuchi Y, Yamanaka H, Nakanishi T, Hayashi T, Ji B, Higuchi M, Suhara T, Imamura K, Araie M, Watanabe Y, Onoe H, Hara H (2012) An alteration in the lateral geniculate nucleus of experimental glaucoma monkeys: in vivo positron emission tomography imaging of glial activation. PLoS One 7(1):e30526
51. Jones EG, Hendry SH (1989) Differential calcium binding protein immunoreactivity distinguishes classes of relay neurons in monkey thalamic nuclei. Eur J Neurosci 1(3):222–246
52. Johnson JK, Casagrande VA (1995) Distribution of calcium-binding proteins within the parallel visual pathways of a primate (*Galago crassicaudatus*). J Comp Neurol 356(2):238–260. doi:10.1002/cne.903560208
53. Le Vay S (1971) On the neurons and synapses of the lateral geniculate nucleus of the monkey, and the effects of eye enucleation. Z Zellforsch Mikrosk Anat 113(3):396–419
54. Somogyi J, Eysel U, Hamori J (1987) A quantitative study of morphological reorganization following chronic optic deafferentation in the adult cat dorsal lateral geniculate nucleus. J Comp Neurol 255(3):341–350. doi:10.1002/cne.902550303
55. Salt TE (1986) Mediation of thalamic sensory input by both NMDA receptors and non-NMDA receptors. Nature 322(6076):263–265. doi:10.1038/322263a0
56. Salt TE (1987) Excitatory amino acid receptors and synaptic transmission in the rat ventrobasal thalamus. J Physiol 391:499–510
57. Scharfman HE, Lu SM, Guido W, Adams PR, Sherman SM (1990) N-methyl-D-aspartate receptors contribute to excitatory postsynaptic potentials of cat lateral geniculate neurons recorded in thalamic slices. Proc Natl Acad Sci U S A 87(12):4548–4552

58. Jones EG, Tighilet B, Tran BV, Huntsman MM (1998) Nucleus- and cell-specific expression of NMDA and non-NMDA receptor subunits in monkey thalamus. J Comp Neurol 397(3):371–393
59. Yucel YH, Gupta N, Zhang Q, Mizisin AP, Kalichman MW, Weinreb RN (2006) Memantine protects neurons from shrinkage in the lateral geniculate nucleus in experimental glaucoma. Arch Ophthalmol 124(2):217–225. doi:10.1001/archopht.124.2.217
60. Ito Y, Nakamura S, Tanaka H, Shimazawa M, Araie M, Hara H (2008) Memantine protects against secondary neuronal degeneration in lateral geniculate nucleus and superior colliculus after retinal damage in mice. CNS Neurosci Ther 14(3):192–202. doi:10.1111/j.1755-5949.2008.00050.x
61. Lipton SA, Singel DJ, Stamler JS (1994) Nitric oxide in the central nervous system. Prog Brain Res 103:359–364
62. Wang X, Sam-Wah Tay S, Ng YK (2000) Nitric oxide, microglial activities and neuronal cell death in the lateral geniculate nucleus of glaucomatous rats. Brain Res 878(1–2):136–147
63. Nucci C, Morrone L, Rombola L, Nistico R, Piccirilli S, Cerulli L (2003) Multifaceted roles of nitric oxide in the lateral geniculate nucleus: from visual signal transduction to neuronal apoptosis. Toxicol Lett 139(2–3):163–173
64. Ilieva EV, Ayala V, Jove M, Dalfo E, Cacabelos D, Povedano M, Bellmunt MJ, Ferrer I, Pamplona R, Portero-Otin M (2007) Oxidative and endoplasmic reticulum stress interplay in sporadic amyotrophic lateral sclerosis. Brain 130(Pt 12):3111–3123
65. Okada K, Minamino T, Tsukamoto Y, Liao Y, Tsukamoto O, Takashima S, Hirata A, Fujita M, Nagamachi Y, Nakatani T, Yutani C, Ozawa K, Ogawa S, Tomoike H, Hori M, Kitakaze M (2004) Prolonged endoplasmic reticulum stress in hypertrophic and failing heart after aortic constriction: possible contribution of endoplasmic reticulum stress to cardiac myocyte apoptosis. Circulation 110(6):705–712
66. Kim I, Xu W, Reed JC (2008) Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. Nat Rev Drug Discov 7(12):1013–1030
67. Ito Y, Nakamura S, Tanaka H, Tsuruma K, Shimazawa M, Araie M, Hara H (2010) Lomerizine, a Ca^{2+} channel blocker, protects against neuronal degeneration within the visual center of the brain after retinal damage in mice. CNS Neurosci Ther 16(2):103–114. doi:10.1111/j.1755-5949.2009.00081.x
68. Ito Y, Shimazawa M, Inokuchi Y, Yamanaka H, Tsuruma K, Imamura K, Onoe H, Watanabe Y, Aihara M, Araie M, Hara H (2011) Involvement of endoplasmic reticulum stress on neuronal cell death in the lateral geniculate nucleus in the monkey glaucoma model. Eur J Neurosci 33(5):843–855. doi:10.1111/j.1460-9568.2010.07578.x
69. Srivastava SP, Kumar KU, Kaufman RJ (1998) Phosphorylation of eukaryotic translation initiation factor 2 mediates apoptosis in response to activation of the double-stranded RNA-dependent protein kinase. J Biol Chem 273(4):2416–2423
70. Martin LJ, Kaiser A, Yu JW, Natale JE, Al-Abdulla NA (2001) Injury-induced apoptosis of neurons in adult brain is mediated by p53-dependent and p53-independent pathways and requires Bax. J Comp Neurol 433(3):299–311
71. Martin LJ, Price AC, McClendon KB, Al-Abdulla NA, Subramaniam JR, Wong PC, Liu Z (2003) Early events of target deprivation/axotomy-induced neuronal apoptosis in vivo: oxidative stress, DNA damage, p53 phosphorylation and subcellular redistribution of death proteins. J Neurochem 85(1):234–247
72. Repici M, Atzori C, Migheli A, Vercelli A (2003) Molecular mechanisms of neuronal death in the dorsal lateral geniculate nucleus following visual cortical lesions. Neuroscience 117(4):859–867
73. Shimazawa M, Ito Y, Inokuchi Y, Hara H (2007) Involvement of double-stranded RNA-dependent protein kinase in ER stress-induced retinal neuron damage. Invest Ophthalmol Vis Sci 48(8):3729–3736. doi:10.1167/iovs.06-1122

74. Kudo T, Kanemoto S, Hara H, Morimoto N, Morihara T, Kimura R, Tabira T, Imaizumi K, Takeda M (2008) A molecular chaperone inducer protects neurons from ER stress. Cell Death Differ 15(2):364–375. doi:10.1038/sj.cdd.4402276
75. Oida Y, Izuta H, Oyagi A, Shimazawa M, Kudo T, Imaizumi K, Hara H (2008) Induction of BiP, an ER-resident protein, prevents the neuronal death induced by transient forebrain ischemia in gerbil. Brain Res 1208:217–224. doi:10.1016/j.brainres.2008.02.068
76. Inokuchi Y, Nakajima Y, Shimazawa M, Kurita T, Kubo M, Saito A, Sajiki H, Kudo T, Aihara M, Imaizumi K, Araie M, Hara H (2009) Effect of an inducer of BiP, a molecular chaperone, on endoplasmic reticulum (ER) stress-induced retinal cell death. Invest Ophthalmol Vis Sci 50(1):334–344. doi:10.1167/iovs.08-2123
77. Lee YK, Brewer JW, Hellman R, Hendershot LM (1999) BiP and immunoglobulin light chain cooperate to control the folding of heavy chain and ensure the fidelity of immunoglobulin assembly. Mol Biol Cell 10(7):2209–2219
78. van de Put FH, Elliott AC (1997) The endoplasmic reticulum can act as a functional Ca^{2+} store in all subcellular regions of the pancreatic acinar cell. J Biol Chem 272(44):27764–27770
79. Lievremont JP, Rizzuto R, Hendershot L, Meldolesi J (1997) BiP, a major chaperone protein of the endoplasmic reticulum lumen, plays a direct and important role in the storage of the rapidly exchanging pool of Ca^{2+}. J Biol Chem 272(49):30873–30879
80. Helenius A (1994) How N-linked oligosaccharides affect glycoprotein folding in the endoplasmic reticulum. Mol Biol Cell 5(3):253–265
81. Kuznetsov G, Chen LB, Nigam SK (1997) Multiple molecular chaperones complex with misfolded large oligomeric glycoproteins in the endoplasmic reticulum. J Biol Chem 272 (5):3057–3063
82. Klausner RD, Sitia R (1990) Protein degradation in the endoplasmic reticulum. Cell 62 (4):611–614
83. Blond-Elguindi S, Cwirla SE, Dower WJ, Lipshutz RJ, Sprang SR, Sambrook JF, Gething MJ (1993) Affinity panning of a library of peptides displayed on bacteriophages reveals the binding specificity of BiP. Cell 75(4):717–728
84. Knarr G, Gething MJ, Modrow S, Buchner J (1995) BiP binding sequences in antibodies. J Biol Chem 270(46):27589–27594
85. Knarr G, Modrow S, Todd A, Gething MJ, Buchner J (1999) BiP-binding sequences in HIV gp160. Implications for the binding specificity of bip. J Biol Chem 274(42):29850–29857
86. Brodsky JL, Werner ED, Dubas ME, Goeckeler JL, Kruse KB, McCracken AA (1999) The requirement for molecular chaperones during endoplasmic reticulum-associated protein degradation demonstrates that protein export and import are mechanistically distinct. J Biol Chem 274(6):3453–3460
87. Meerovitch K, Wing S, Goltzman D (1998) Proparathyroid hormone-related protein is associated with the chaperone protein BiP and undergoes proteasome-mediated degradation. J Biol Chem 273(33):21025–21030
88. 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 (1999) Presenilin-1 mutations downregulate the signalling pathway of the unfolded-protein response. Nat Cell Biol 1(8):479–485. doi:10.1038/70265
89. Yu Z, Luo H, Fu W, 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(2):302–314. doi:10.1006/exnr.1998.7002, S0014-4886(98)97002-9 [pii]
90. Rao RV, Peel A, Logvinova A, del Rio G, Hermel E, Yokota T, Goldsmith PC, Ellerby LM, Ellerby HM, Bredesen DE (2002) Coupling endoplasmic reticulum stress to the cell death program: role of the ER chaperone GRP78. FEBS Lett 514(2–3):122–128, S0014579302022895 [pii]

91. Reddy RK, Mao C, Baumeister P, Austin RC, Kaufman RJ, Lee AS (2003) Endoplasmic reticulum chaperone protein GRP78 protects cells from apoptosis induced by topoisomerase inhibitors: role of ATP binding site in suppression of caspase-7 activation. J Biol Chem 278 (23):20915–20924
92. Weil M, Jacobson MD, Coles HS, Davies TJ, Gardner RL, Raff KD, Raff MC (1996) Constitutive expression of the machinery for programmed cell death. J Cell Biol 133 (5):1053–1059
93. Taylor J, Gatchalian CL, Keen G, Rubin LL (1997) Apoptosis in cerebellar granule neurones: involvement of interleukin-1 beta converting enzyme-like proteases. J Neurochem 68 (4):1598–1605
94. Inokuchi Y, Shimazawa M, Nakajima Y, Suemori S, Mishima S, Hara H (2006) Brazilian green propolis protects against retinal damage in vitro and in vivo. Evid Based Complement Alternat Med 3(1):71–77. doi:10.1093/ecam/nek005
95. Shimazawa M, Inokuchi Y, Ito Y, Murata H, Aihara M, Miura M, Araie M, Hara H (2007) Involvement of ER stress in retinal cell death. Mol Vis 13:578–587
96. Feng G, Mellor RH, Bernstein M, Keller-Peck C, Nguyen QT, Wallace M, Nerbonne JM, Lichtman JW, Sanes JR (2000) Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. Neuron 28(1):41–51
97. Henneberry RC, Novelli A, Cox JA, Lysko PG (1989) Neurotoxicity at the N-methyl-D-aspartate receptor in energy-compromised neurons. An hypothesis for cell death in aging and disease. Ann N Y Acad Sci 568:225–233
98. Ubeda M, Habener JF (2000) CHOP gene expression in response to endoplasmic-reticular stress requires NFY interaction with different domains of a conserved DNA-binding element. Nucleic Acids Res 28(24):4987–4997
99. Foti DM, Welihinda A, Kaufman RJ, Lee AS (1999) Conservation and divergence of the yeast and mammalian unfolded protein response. Activation of specific mammalian endoplasmic reticulum stress element of the grp78/BiP promoter by yeast Hac1. J Biol Chem 274 (43):30402–30409