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A Golgi fragmentation pathway in neurodegeneration

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Abstract

The Golgi apparatus processes intracellular proteins, but undergoes disassembly and fragmentation during apoptosis in several neurodegenerative disorders such as amyotrophic lateral sclerosis and Alzheimer's disease. It is well known that other cytoplasmic organelles play important roles in cell death pathways. Thus, we hypothesized that Golgi fragmentation might participate in transduction of cell death signals.

Here, we found that Golgi fragmentation and dispersal precede neuronal cell death triggered by excitotoxins, oxidative/nitrosative insults, or ER stress. Pharmacological intervention or overexpression of the C-terminal fragment of Grasp65, a Golgi-associated protein, inhibits fragmentation and decreases or delays neuronal cell death. Inhibition of mitochondrial or ER cell death pathways also decreases Golgi fragmentation, indicating crosstalk between organelles, and suggesting that the Golgi may be a common downstream-effector of cell death. Taken together, these findings implicate the Golgi as a sensor of stress signals in cell death pathways.

Keywords

Golgi fragmentation; ER stress; apoptosis; mitochondria; Alzheimer's disease; amyotrophic lateral sclerosis; excitotoxicity; NMDA; oxidative stress; nitrosative stress

Introduction

The Golgi apparatus is a cytoplasmic organelle involved in the transport, processing, and targeting of proteins synthesized in the rough endoplasmic reticulum and destined for the secretory pathway. In normal cells, the Golgi apparatus is composed of a series of flattened, parallel, interconnected cisternae organized around the microtubule-organizing center in the perinuclear region. The Golgi apparatus was originally thought to be a static organelle, but it

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is actually a highly dynamic structure. Examples of the Golgi's dynamic behavior include its reversible disassembly during mitosis, when the Golgi apparatus fragments to produce clusters of vesicles that disperse throughout the cytoplasm (Robbins and Gonatas, 1964; Warren, 1993). These mitotic Golgi fragments are equally partitioned into the daughter cells. Upon exit from the mitotic program, the perinuclear Golgi apparatus is reconstituted simultaneously with the reformation of the nuclear envelope. Recent reports show that inhibition of Golgi fragmentation prevents entry into mitosis, suggesting that Golgi fragmentation is not merely a response to mitosis but a causal event in the process (Sutterlin et al., 2002).

It has also been reported that during apoptotic cell death of non-neuronal cells, Golgi stacks disperse and disassemble into tubulovesicular clusters, a process that bears some similarity to mitotic disassembly of the Golgi complex (Chiu et al., 2002; Lane et al., 2002; Machamer, 2003). Moreover, inhibiting caspase-mediated cleavage of the Golgi-associated protein Golgin-160 partially prevented cell death of these non-neuronal cells (Hicks and Machamer, 2005; Maag et al., 2005). Since the Golgi apparatus is involved in numerous important functions, such as the transport, processing, and targeting of proteins synthesized in the endoplasmic reticulum (ER), quality control of proteins in the Golgi apparatus and ER must be stringent to ensure appropriate cellular function. Thus, we reasoned that fragmentation of Golgi during cell death might have detrimental effects and lead to dysfunction of the cytoplasmic machinery in neurons as well as in non-neuronal cells. Along these lines, fragmentation of the Golgi apparatus has been reported in vivo in several human neurodegenerative diseases, including Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) (Gonatas et al., 2006). Heretofore, however, it was not known if Golgi participate in a causal manner in the signaling cascade of cell death pathways. Here we present evidence that the Golgi apparatus is a sensor for controlling entry into apoptosis.

Experimental procedures

Cell culture

Cerebrocortical neurons were isolated from embryonic day 15 or 16 rats and cultured as described (Budd et al., 2000). These cultures contain a mixture of cell types, including neurons, astrocytes, and microglia.

DNA constructs and transfection

DsRed2-mito expression constructs were purchased from Clontech. Mitofusin 1 (Mfn1) and Bax inhibitor-1 (BI-1) expression constructs were provided by M.T. Fuller (Stanford University) and John C. Reed (Burnham Institute for Medical Research), respectively. Grasp65 expression constructs were produced in the laboratory of one of the authors (V.M.). The expression construct for protein disulfide isomerase (PDI) has been described previously (Ko et al., 2002).

Cultures were transfected after 14-17 days in vitro using Lipofectamine 2000 (Invitrogen). Transfection efficiency was 5 to 10%. Based on prior work, during co-transfection, if the labeled construct was successfully transduced then so was the second unlabeled construct (Mao et al., 1999). Experiments were performed 2 days after transfection.

Drug exposure

Cultures were exposed to *N*-methyl-D-aspartate (NMDA, Sigma) for 20 min with 5 μ M glycine as described previously (Bonfoco et al., 1995; Budd et al., 2000), followed by replacement with growth medium or imaging buffer. S-nitrosocysteine (SNOC) was prepared as described previously (Lei et al., 1992). D-(-)-2-Amino-5-phosphonovaleric acid (D-APV, Tocris) was applied to cultures at a final concentration of 100 μ M prior to NMDA application. The NOS

inhibitor N-nitro-L-arginine was added to cultures at a final concentration of 1 mM. Thapsigargin (Calbiochem) was dissolved in DMSO and applied to neurons in their growth medium. The protein kinase A (PKA) antagonists, H89 and PKI (Calbiochem), were dissolved in DMSO and added to cultures at a final concentration of 1 mM and 1 μ M, respectively.

Immunocytochemistry

Cerebrocortical neurons were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 15 min, washed, and permeabilized with 0.2% Triton X-100 in PBS for 5 min. After another brief wash, fixed cells were blocked with PBS containing 1% BSA for 1 hour at room temperature. Primary antibody incubations were performed using anti-c-myc polyclonal antibody (Santa Cruz) or anti-microtubule associated protein-2 (MAP-2) monoclonal antibody (Sigma) in PBS containing 1% BSA for 14-16 hours at 4°C. After washing with PBS, the cells were incubated with anti-mouse or anti-rabbit antibodies conjugated with Alexa 405/488/594 for 1 hour at room temperature.

For live-cell imaging, growth medium was replaced with imaging buffer (pH 7.4) containing (in mM): 120 NaCl, 5.4 KCl, 0.8 MgCl₂, 1.8 CaCl₂, 15 D-(+)-glucose, and 5 HEPES. Cultures were maintained at 37° C on a heated microscope stage for time-lapse imaging.

Fluorescence deconvolution microscopy for assessing mitochondrial fragmentation, Golgi fragmentation/dispersal, and neuronal cell death

To observe mitochondrial morphology, cultures were transfected with the mitochondrial marker, DsRed-mito. Mitochondrial fission was detected by fragmentation of a single tubular mitochondrion into multiple, shorter punctiform organelles under fluorescence deconvolution microscopy (Youle and Karbowski, 2005). Golgi fragmentation and dispersal was assessed by observing dispersed, punctated Golgi-green fluorescence protein (GFP) under fluorescence deconvolution microscopy. Cells were scored in a masked fashion. Neuronal cell death was assessed by chromatin condensation after Hoechst staining. Typically, several hundred cells were scored in each experiment. Images were acquired using an Axiovert 100M microscope (Carl Zeiss) and Slidebook software (Intelligent Imaging Innovations). Deconvolution was performed using the constrained iterative algorithm in Slidebook.

Statistical Methods

Statistical significance was determined via a Student's t-test for single comparisons or an analysis of variance (ANOVA) followed by a post hoc Schéffe's test for multiple comparisons.

Results

Fragmentation of the Golgi apparatus during neuronal injury

Given the potential importance of excitotoxins such as the neurotransmitter glutamate in contributing to neurodegenerative disorders (Lipton and Rosenberg, 1994), we initially tested the effects of this pathway on Golgi fragmentation. We found that excessive stimulation of NMDA-sensitive glutamate receptors resulted in neuronal injury preceded by Golgi fragmentation (Fig. 1). For these experiments, cerebrocortical neurons were transfected with a GFP vector fused with a mannosidase II transmembrane domain (Golgi-GFP) to visualize Golgi morphology. In control cells, the Golgi apparatus localized to a compact perinuclear ribbon, as expected. In contrast, after NMDA exposure, the Golgi apparatus fragmented into punctate structures dispersed throughout the cytoplasm (Fig. 1A). We tracked Golgi morphology using fluorescence time-lapse microscopy. This analysis revealed that the Golgi started to fragment about 1 hour after NMDA exposure, and the fragmentation proceeded in a time- and concentration-dependent manner (Figs. 1A, B). D-APV, a specific NMDA receptor

antagonist, prevented NMDA-induced Golgi fragmentation. It is well known that excessive stimulation of NMDA receptors engenders excessive Ca^{2+} influx, leading to neuronal injury and death in part via nitric oxide (NO) generated by neuronal nitric oxide synthase (NOS) (Dawson et al., 1993; Dawson et al., 1991). Here, we found that nitro-L-arginine, a NOS inhibitor, significantly inhibited NMDA-induced Golgi fragmentation, suggesting that NO mediated Golgi fragmentation downstream of NMDA receptor activation. To confirm that NO can induce Golgi fragmentation in cortical neurons, we applied the endogenous NO donor S-nitrosocysteine (SNOC) to the cultures. As expected, SNOC induced Golgi fragmentation (Figs. 1 and 2).

Next, since the Golgi and ER perform interrelated functions in processing and transporting proteins, we tested whether ER stress could induce Golgi fragmentation. For this purpose, we used thapsigargin, an inhibitor of the Ca^{2+} -ATPase in the ER that is known to induce cell death. After exposure to thapsigargin but long before cell death, the Golgi apparatus fragmented and dispersed throughout the cytosol (Fig. 1). The percentage of neurons with fragmented Golgi increased in a dose-dependent fashion (Fig. 2).

Golgi function is intimately related to the integrity of microtubules. Golgi stacks are usually arranged in an interconnected network of cytoplasmic microtubules. Therefore, we next determined if fragmentation of Golgi might be a consequence of disorganization of tubulin during cell death. For this series of experiments, neurons were co-transfected with fluorescently-tagged constructs to specifically label both microtubules and Golgi (pYFP-Tub encodes a fusion protein consisting of yellow fluorescence protein (YFP) and human α -tubulin, while pCFP-Golgi encodes a fusion protein consisting of cyan fluorescence protein (CFP) and the mannosidase II transmembrane domain). In control cells, the normal fibrillar structure of tubulin was observed in neuronal processes. This tubulin structure persisted more than 3 hours after NMDA exposure, a time point when the Golgi had already fragmented and dispersed throughout the cytosol. After 6 to 9 hours, neurons began to degenerate, as evidenced by neurite injury and tubulin disintegration (Fig. 3). These results suggest that Golgi fragmentation precedes tubulin degradation and neuronal injury.

Inhibition of mitochondrial fission prevents Golgi fragmentation after NMDA or SNOC exposure

We next asked if mitochondrial dysfunction, known to play a pivotal role in the pathogenesis of many neurodegenerative disorders, effected Golgi fragmentation. In fact, during some forms of apoptosis, mitochondria are also known to undergo fission and fragmentation (Youle and Karbowski, 2005). The mitochondrial protein Mfn1 is required for mitochondrial fusion, and its overexpression has been shown to prevent mitochondrial fission and neuronal cell death after exposure to NMDA or SNOC; dynamin related protein 1 (Drp1) is involved in mitochondrial fission, and a dominant negative construct (Drp1-DN) can also prevent mitochondrial fission and neuronal cell death after exposure to NMDA or SNOC (Barsoum et al., 2006; Bossy-Wetzel et al., 2003). In the current experiments, we co-transfected neurons with Mfn1 or Drp1-DN plus a mitochondrial label (DsRed-mito, a red fluorescent marker for the mitochondrial inner membrane) or a Golgi marker (Golgi-GFP). The neurons were then exposed to NMDA, SNOC, or thapsigargin. In control transfections lacking Mfn1 or Drp1-DN, mitochondrial fragmentation (particularly evident in the neurites) occurred within 3 hours of exposure to NMDA or SNOC. Mfn1, however, largely prevented this mitochondrial fission (Fig. 4A, C). In contrast to NMDA and SNOC, a 3-hour exposure to thapsigargin induced far less mitochondrial fragmentation. Next, we asked whether inhibition of mitochondrial fragmentation with Mfn1 or Drp1-DN could prevent Golgi fragmentation. Indeed, Mfn1 (or Drp1-DN) decreased Golgi fragmentation after NMDA or SNOC exposure (Figs. 4B, D and data not shown). However, Mfn1 or Drp1-DN did not inhibit Golgi fragmentation and dispersal

evoked by thapsigargin. Since thapsigargin did not induce mitochondrial fragmentation, thapsigargin-induced Golgi fragmentation may represent an independent pathway from that of mitochondria.

Inhibition of the ER stress pathway prevents Golgi fragmentation

Similar to mitochondria, the ER can initiate a cell death pathway following severe ER stress. Hence, we examined whether inhibition of ER stress could prevent Golgi fragmentation. ER proteins such as protein-disulfide isomerase Bax inhibitor 1 (BI-1) and protein-disulfide isomerase (PDI) are known to inhibit this ER pathway and prevent neuronal cell death (Chae et al., 2004; Ko et al., 2002; Tanaka et al., 2000). We found that overexpression of BI-1 manifested little effect by itself but partially prevented Golgi fragmentation and dispersal induced by various insults in cortical neurons (cf Fig. 5 and Fig. 1). This effect was more apparent on preventing dispersal throughout the cell of the normal ribbon-like structure of the Golgi after exposure to these insults (Fig. 5A). Additionally, PDI prevented thapsigargin-induced Golgi fragmentation (Fig. 6) but not NMDA- or SNOC-induced fragmentation (data not shown). This finding is consistent with the recently published finding that PDI is no longer effective after *S*-nitrosylation (due to the addition of either exogenous NO or endogenous NO generated by NMDA stimulation) (Uehara et al., 2006). These data indicate that under these conditions Golgi fragmentation is apparently downstream of the ER stress pathway.

Inhibition of Golgi fragmentation decreases or delays neuronal cell death

Most importantly, we wanted to determine whether Golgi fragmentation pathway is causally involved in cell death. For this purpose, we initially used two pharmacological antagonists of protein kinases, H89 and PKI. H89 inhibits multiple protein kinases, including A (PKA), C (PKC), and D (PKD). Both PKA and PKD have been implicated in the control of Golgi morphology (Jamora et al., 1999; Weinberger et al., 2005). Additionally, H89 is known to abrogate cell cycle-induced Golgi fragmentation (Jamora et al., 1999). We found that treatment with H89 significantly attenuated Golgi fragmentation in cortical neurons after an apoptosis-inducing insult with NMDA (Figs. 7A, B). Moreover, H89 delayed cell death evoked by NMDA when monitored 12 (but not 24) hours after insult. Similarly, we found that the more specific PKA inhibitor, PKI, also inhibited Golgi fragmentation and delayed cell death in cortical neurons after NMDA exposure (Fig. 7A, B). These results suggest that PKA might be involved in Golgi fragmentation associated with apoptotic neuronal cell death. However, no pharmacological agent is perfectly specific, and it is likely that these protein kinase inhibitors manifest pleiotropic effects in addition to those on the Golgi apparatus.

Therefore, we next used a more specific molecular interference to prevent Golgi fragmentation in response to multiple apoptotic insults, including NMDA, SNOC or thapsigargin, and we simultaneously monitored neuronal apoptosis. To interfere with Golgi fragmentation, we used the C-terminal fragment of the Golgi-associated protein Grasp65, which is known to specifically inhibit Golgi fragmentation during mitosis (Sutterlin et al., 2002). We found that Grasp65 could also partially inhibit Golgi fragmentation after an apoptotic insult. For this purpose, we transfected cerebrocortical neurons with a construct expressing the C-terminal fragment of Grasp65 fused to a c-myc tag (Grasp65 Δ 200). In Grasp65 Δ 200-transfected cells, Golgi fragmentation and dispersal was partially inhibited in response to an apoptotic-inducing concentration of NMDA, SNOC or thapsigargin (Figs. 7C, D). Although the Golgi underwent some morphological changes, we found less dispersal from the perinuclear region of punctuated Golgi when scored in a blinded fashion. As a suitable control, we transfected the N-terminal fragment of Grasp65 (Grasp65a.a.1-204), which manifested no significant effect on Golgi fragmentation and dispersal in response to an apoptotic insult when compared to Golgi-GFP plus pcDNA3.1. Importantly, inhibition of Golgi fragmentation/dispersal by Grasp65 Δ 200 (but not Grasp65a.a.1-204) also rescued neurons from cell death. After

Grasp65 Δ 200 transfection, the percentage of neurons with pyknotic, apoptotic nuclei was significantly decreased 12 hours post NMDA or SNOC insult, and at least 24 hours post thapsigargin (Fig. 8). While Grasp65 Δ 200 decreased Golgi fragmentation/dispersal in response to thapsigargin by ~20%, neuronal apoptosis decreased by ~40%; however, our very strict criteria for scoring inhibition of Golgi dispersal may have excluded more subtle effects and could therefore have easily underestimated the percentage of cells affected. Overall, these results are consistent with the notion that the primary cell death pathway after thapsigargin insult involves Golgi fragmentation/dispersal. In contrast, after NMDA or SNOC exposure, although inhibition of Golgi fragmentation/dispersal can delay neuronal apoptosis, other cell death pathways can subsequently intervene.

Discussion

Our findings suggest that the Golgi apparatus can sense and transduce death signals. Along these lines, Golgi fragmentation and dispersal, resulting from exposure to death signals, may be a harbinger for subsequent neuronal apoptosis. Similar to the ER and mitochondria, the Golgi complex may thus initiate stress signaling through its own unique molecular machinery. We found that several types of neuronal insult induce Golgi fragmentation, including excitotoxicity, reactive oxygen or nitrogen species, and ER stress. Importantly, pharmacological or molecular inhibition of Golgi fragmentation and dispersal decreased or delayed apoptotic-like cell death, implicating a causal effect of the Golgi apparatus in these cell death pathways. We also found that molecular interference with mitochondrial- or ER-mediated apoptotic pathways partially abrogates Golgi fragmentation and neuronal cell death, suggesting that these other organelles are upstream in the cell death pathway in response to their apoptotic effectors (Fig. 9).

Since the ER and Golgi are closely linked to one another, both morphologically and functionally, it may be difficult to discriminate between ER- and Golgi-mediated pathways to cell death. Indeed, the Golgi undergo dispersal/fragmentation in response to the ER-stress inducer, thapsigargin. It is also possible that NMDA and SNOC exposure might impact ER function, resulting in Golgi fragmentation. However, during oxidative stress, the cell death-related protein annexin II was recently reported to be phosphorylated by the Src kinase Lyn. Phosphorylated annexin II is subsequently translocated from the Golgi to the ER (Matsuda et al., 2006). This finding indicates that the Golgi can initiate cell-death signaling upstream from the ER, as also observed in the present study.

Previously, Golgi fragmentation had been reported to occur in non-neuronal cell types after various forms of stress (Chiu et al., 2002; Lane et al., 2002; Machamer, 2003). Traditionally, apoptotic Golgi disassembly has been considered a late event, providing disposal of the Golgi apparatus. Since Golgi stacks are usually arranged as an interconnected network of cytoplasmic microtubules and a close relationship exists between Golgi elements and microtubules, fragmentation of the Golgi was considered to be merely a consequence of tubulin degradation during cell death. However, our findings reveal that the process of Golgi fragmentation/dispersal is an early event in neuronal cell death and is initiated prior to tubulin degeneration, which is observed in the middle stages of apoptosis. Moreover, since prevention of Golgi fragmentation/dispersal at an early stage partially inhibited cell death, fragmentation of the Golgi apparatus appears to be an early event and not a consequence of cytoskeletal degradation during cell death.

Fragmentation and dispersal of the Golgi apparatus is also known to occur in vivo in the human nervous system during various neurodegenerative diseases such as ALS, AD, corticobasal degeneration, Creutzfeldt-Jakob disease, and spinocerebellar ataxia type 2 (SCA2) (Gonatas et al., 2006). Additionally, in an animal model of ALS represented by the mutant SOD1

transgenic mouse, the Golgi apparatus fragments prior to the onset of paralysis (Mourelatos et al., 1994; Stieber et al., 2004). In cells expressing mutant SOD1, fragmentation of the Golgi apparatus is associated with dysfunction of the secretory pathway (Stieber et al., 2004). Golgi fragmentation might thus cause dysfunction of cellular processes, such as protein trafficking and secretion.

Previous findings have suggested that the Golgi apparatus contains known elements of the cell death machinery. For example, pro-apoptotic caspase-2 has been localized to the Golgi apparatus (Bennett et al., 1998; Mancini et al., 2000; Schneider-Brachert et al., 2004). Hence, caspase-2 might initiate apoptosis after irreparable stress to the secretory pathway. Several reports implicate caspase-mediated cleavage of Golgi structural proteins, such as Grasp65, golgin-160, and p115, in causing such damage (Chiu et al., 2002; Lane et al., 2002; Maag et al., 2005). Taken together with our new results, Golgi fragmentation and dispersal appears to be causally related to neurodegeneration. Finally, it has been suggested that aberrant entry into the cell cycle can trigger neuronal apoptosis. Since mature neurons cannot undergo mitosis, the initiation of cell cycle events may contribute to an apoptotic pathway (Farinelli and Greene, 1996; Olsen et al., 2006). Along these lines, our results suggest that Golgi fragmentation and dispersal, which normally occur during mitosis, may contribute to neuronal apoptosis in such situations.

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Abbreviations

AD, Alzheimer's disease
 ALS, amyotrophic lateral sclerosis
 D-APV, D-(-)-2-amino-5-phosphonovaleric acid
 BI-1, Bax inhibitor-1
 CFP, cyan fluorescence protein
 Drp1, dynamin related protein 1
 ER, endoplasmic reticulum
 GFP, green fluorescence protein
 MAP-2, microtubule associated protein-2
 Mfn1, Mitofusin 1
 NMDA, N-methyl-D-aspartate
 NO, nitric oxide
 NOS, neuronal NO synthase
 PBS, phosphate-buffered saline
 PDI, protein disulfide isomerase
 PKA, protein kinase A
 PKC, protein kinase C
 PKD, protein kinase D
 PKI, protein kinase A inhibitor
 SCA2, spinocerebellar ataxia type 2
 SNOC, S-nitrosocysteine
 S.E.M, standard error of the mean
 YFP, yellow fluorescence protein

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Fig. 1. Golgi fragmentation during neuronal injury in primary cerebrocortical cultures. (A) Morphology of the Golgi apparatus in primary cultures of cortical neurons 3 hours after exposure to 50 μ M NMDA, 200 μ M SNOC, or 0.1 μ g/ml thapsigargin. Golgi stained with Golgi-GFP (green) and neurons with MAP-2 antibody (red). (B) Time-lapse deconvolution microscopy of the Golgi apparatus undergoing fragmentation in neurons after exposure to 50 μ M NMDA.

Fig. 2. Percentage of neurons with fragmented Golgi 3 hours after exposure to NMDA, SNOC, or Thapsigargin. Golgi fragmentation was blindly scored by assessing punctated, dispersed Golgi-GFP signal throughout the cytoplasm. Values are mean + S.E.M. from $N = 5$ experiments with >100 neurons counted in each experiment ($*p \leq 0.01$).

Fig. 3. Morphology of tubulin and Golgi after drug treatment in primary cultures of cortical neurons. Tubulin was stained with YFP-tubulin (yellow-green) and Golgi with CFP-mannosidase II (blue) 3, 6, or 9 h after exposure to NMDA. Scale bar, 20 μ m.

Fig. 4. Inhibition of mitochondrial fission by Mfn1 prevents Golgi fragmentation in cortical neurons. (A) Mitochondrial morphology 3 h after insult. Cortical neurons were transfected with DsRed-mito plus pcDNA3.1 (Cont.) or Mfn and subsequently exposed to 50 μ M NMDA, 200 μ M SNOC, or 0.1 μ g/ml thapsigargin. Arrowheads indicate fragmented mitochondria in neurites. (B) Golgi morphology after 50 μ M NMDA, 200 μ M SNOC, or 0.1 μ g/ml thapsigargin. Cortical neurons were transfected with Golgi-GFP and Mfn1. Neurons were stained with MAP-2 antibody (red). Scale bar, 20 μ m. (C) Percentage of neurons undergoing mitochondrial fragmentation after insult. Cortical neurons co-transfected with DsRed-mito plus pcDNA3.1 (Cont.) or Mfn1 were exposed to 50 μ M NMDA, 200 μ M SNOC or 0.1 μ g/ml thapsigargin,

and evaluated for mitochondrial morphology. Values are mean + S.E.M. for $N = 5$ experiments ($**p \leq 0.05$). (D) Percentage of neurons undergoing Golgi fragmentation after insult. Cortical neurons co-transfected with Golgi-GFP plus pcDNA3.1 (Cont.) or Mfn1 were exposed to 50 μM NMDA, 200 μM SNOC or 0.1 $\mu\text{g/ml}$ thapsigargin, and evaluated for Golgi morphology. Values are mean + S.E.M. for $N = 5$ experiments ($*p \leq 0.01$; $**p \leq 0.05$).

Fig. 5.

Inhibition of ER stress by BI-1 decreases Golgi fragmentation and dispersal. (A) Golgi morphology 3 h after exposure to 50 μ M NMDA, 200 μ M SNOC, or 0.1 μ g/ml thapsigargin. Cortical neurons co-transfected with Golgi-GFP and myc-BI-1 were immunostained with anti-c-myc (red) and anti-MAP-2 (blue). Scale bar, 20 μ m. (B) Percentage of neurons undergoing Golgi fragmentation/dispersal after exposure to NMDA, SNOC, and thapsigargin. Cortical neurons co-transfected with Golgi-GFP plus pcDNA3.1 or BI-1 were exposed to drugs and evaluated for Golgi morphology. Values are mean + S.E.M. from $N = 5$ experiments (* $p \leq 0.01$; ** $p \leq 0.05$).

Fig. 6. Inhibition of ER stress by PDI decreases Golgi fragmentation and dispersal. (A) Golgi morphology 3 h after 0.1 µg/ml thapsigargin exposure. Cortical neurons co-transfected with Golgi-GFP and myc-PDI were immunostained with anti-c-myc (red) and anti-MAP-2 (blue). Scale bar, 20 µm. (B) Percentage of neurons undergoing Golgi fragmentation after exposure to thapsigargin. Cortical neurons co-transfected with Golgi-GFP plus pcDNA3.1 or PDI were exposed to thapsigargin and evaluated for Golgi morphology. Values are mean + S.E.M. from $N = 5$ experiments ($*p \leq 0.01$).

Fig. 7.

Inhibition of Golgi fragmentation and dispersal by protein kinase inhibitors or Grasp65Δ200. (A) Percentage of neurons with fragmented Golgi 3 hours after exposure to NMDA. Cortical neurons were exposed to NMDA with or without the protein kinase inhibitors, H89 or PKI. Values are mean + S.E.M. for $N = 5$ experiments ($*p \leq 0.01$). (B) Percentage of neurons with apoptotic nuclei after exposure to NMDA. Cortical neurons were exposed to NMDA with or without the protein kinase inhibitors. Then, 12 or 24 h later, cells were fixed and nuclei stained with Hoechst. Values are mean + S.E.M. from $N = 5$ experiments ($*p \leq 0.01$). (C) Golgi morphology 3 hours after 50 μM NMDA, 200 μM SNOC, or 0.1 μg/ml thapsigargin. Cortical neurons co-transfected with Golgi-GFP and myc-tagged Grasp65Δ200 were immunostained

with anti-c-myc (red) and anti-MAP-2 (blue). Scale bar, 20 μm . (D) Percentage of neurons undergoing Golgi fragmentation and dispersal after exposure to NMDA, SNOC, or thapsigargin. Cortical neurons co-transfected with Golgi-GFP plus pcDNA3.1 or Grasp65 constructs (a.a.1-204 or $\Delta 200$) were exposed to insult and evaluated for Golgi morphology. Values are mean + S.E.M. for $N = 5$ experiments ($*p \leq 0.01$).

Fig. 8.

Inhibition of Golgi fragmentation decreases or delays neuronal cell death. (A) Golgi and nuclear morphology after exposure to 50 μ M NMDA. Cortical neurons were co-transfected with Golgi-GFP plus pCND3.1 or Grasp65 Δ 200 and exposed to NMDA. Nuclear morphology was assessed by Hoechst staining (blue), with condensed nuclei (arrow) signifying apoptosis. Scale bar, 20 μ m. (B) Percentage of neurons with apoptotic nuclei after exposure to 50 μ M NMDA, 200 μ M SNOC, or 0.1 μ g/ml thapsigargin. Cortical neurons co-transfected with Golgi-GFP plus pCND3.1 or Grasp65 Δ 200 were exposed to insult. Then, 12 or 24 h later, cells were fixed and nuclei stained with Hoechst. Values are mean $\pm$ S.E.M. from $N = 5$ experiments (* $p \leq 0.01$; ** $p \leq 0.05$).

Fig. 9.

Crosstalk between subcellular organelles during neuronal cell death. Organelles sense damage to neurons and integrate apoptotic signaling. Each organelle can activate specific signal transduction pathways to promote or prevent cell death. Data presented here show that inhibition of the Golgi-mediated cell death pathway only delays apoptosis after NMDA or SNOC insult because other death pathways (e.g., mitochondrial) can intervene. In contrast, interruption of the Golgi pathway produces a more long-lasting inhibition of thapsigargin-initiated damage. Note that the ER can also directly communicate with mitochondria in apoptotic signaling using Ca^{2+} as a messenger (Breckenridge et al., 2003; Scorrano, 2003; Scorrano et al., 2003).