Pretreatment with inducers of ER molecular chaperones protects epithelial cells subjected to ATP depletion

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Bush, Kevin T., Sathish K. George, Ping L. Zhang, and Sanjay K. Nigam. Pretreatment with inducers of ER molecular chaperones protects epithelial cells subjected to ATP depletion. Am. J. Physiol. 277 (Renal Physiol. 46): F211–F218, 1999.—We have investigated the potential cytoprotective role of endoplasmic reticulum (ER) molecular chaperones in a cultured cell model of renal ischemia. Madin-Darby canine kidney (MDCK) cells were pretreated with tunicamycin (an inducer of ER but not cytosolic molecular chaperones) for 12–16 h, followed by 6 h of ATP depletion. A rapid and severe depletion of cellular ATP was noted in both control and tunicamycin-treated cells. Trypan blue exclusion assays indicated that pretreatment of MDCK cells with tunicamycin reduced ATP depletion-induced cell damage by ~80% compared with nonpretreated controls. This apparent cytoprotective effect was also found following pretreatment with another inducer of ER molecular chaperones (i.e., A23187). For example, A23187 was found to reduce lactate dehydrogenase release by ~50% compared with untreated controls, whereas E-64, a cysteine protease inhibitor which may affect degradation of some proteins in the ER, had little or no effect on cell injury. Moreover, a fluorescent assay confirmed the marked reduction in cell damage following ATP depletion (up to 80% reduction in tunicamycin-pretreated cells). Together, these findings are consistent with the notion that induction of ER molecular chaperones leads to the acquisition of cytoprotection in the face of ATP depletion. However, inhibition of protein translation by cycloheximide was found to only partially attenuate the observed cytoprotective effect, raising the possibility that other, as yet to be identified, nonprotein synthesis-dependent mechanisms may also play a role in the observed cytoprotection.

Heat-shock proteins (Hsps) were originally identified as a group of cellular proteins whose transcription was induced following the exposure of cells to high temperature (14, 38). A variety of cell “stresses” (e.g., hypoxia, alcohol, heavy metals, and anoxia), which appear to cause the accumulation of misfolded or abnormal protein, can also induce the Hsps. Many Hsps, particularly of the Hsp70 family of proteins, are thought to function as molecular chaperones involved in the normal folding, assembly, and/or degradation of cellular proteins (2, 6, 10, 38). It is this chaperone function of the Hsps that appears to be important to the cellular response to stress. Hsps are believed to bind to misfolded or abnormal proteins and prevent their aggregation, either by rescuing such proteins from irreversible damage (2, 6, 10, 12, 14, 32, 38) or by increasing their susceptibility to proteolytic attack (12). Regardless, increased levels of Hsps in response to stresses that damage and/or denature cellular protein or perturb protein biosynthetic processes appear to be important for cell survival.

Although a wealth of data support an important role for cytosolic Hsps, in particular Hsp70, in cell survival after stress (reviewed in Ref. 32), the role of analogous proteins found in other cellular compartments, including the endoplasmic reticulum (ER), remains unclear. In a recent study, whole kidney ischemia and reperfusion as well as ATP depletion of cultured renal and thyroid epithelial cells was found to increase not only the expression of cytosolic Hsps (e.g., Hsp70), but also the expression of ER molecular chaperones (e.g., BiP, grp94, and ERp72) (17). Because the accumulation of unfolded or misfolded proteins in the ER is associated with the induction of the ER molecular chaperones (5, 20, 28) and because proper folding in the ER requires ATP, one major effect of ATP depletion, and perhaps whole organ ischemia as well, is likely to be the perturbation of normal protein processing within the ER leading to the induction of ER molecular chaperones. Furthermore, because a number of proteins whose functions are thought to be disrupted by ischemia (i.e., cell adhesion molecules, intercellular junction proteins, integrins, extracellular matrix proteins) are folded and assembled in the ER and molecular chaperones of the ER are believed to bind nascent polypeptides and catalyze the folding and/or assembly of proteins transiting this organelle (10, 14, 29, 38), the interesting possibility exists that ER molecular chaperones could play an important role in both the cellular response to ischemia and recovery from an ischemic insult.

Therefore, we investigated the possible cytoprotective role of ER molecular chaperones in protecting cells against damage due to ATP depletion. Pretreatment of Madin-Darby canine kidney (MDCK) cells with agents that selectively induce ER molecular chaperones (but do not affect the expression of cytosolic Hsp70) was found to significantly increase cell viability (i.e., reduced cell membrane damage) (4) following ATP depletion.

Experimental Procedures

Reagents and chemicals. The cDNA probe for BiP was kindly provided by Dr. Mary-Jane Gething (Melbourne University). A cDNA probe which contains the entire coding sequence for a human heat-shock protein of 70 kDa was from ATCC (Rockville, MD). Tunicamycin, A23187, cycloheximide, and E-64 were from Calbiochem (La Jolla, CA). Antimycin A was from Sigma (St. Louis, MO). The lactate dehydrogenase (LDH) cytotoxicity detection kit was from Boehringer Mannheim (Indianapolis, IN). The fluorescent Live/Dead viability-cytotoxicity kit was from Molecular Probes (Eugene, OR).
Pretreatment with inducers of ER molecular chaperones. Confluent monolayers of MDCK cells growing in Dulbecco’s modified Eagle’s medium (DMEM) containing 5% heat-inactivated fetal calf serum (FCS) and 1× concentration of a penicillin-streptomycin mixture were rinsed twice in Dulbecco’s phosphate-buffered saline (DPBS) and then incubated for 3–16 h in fresh DMEM with or without various chemical agents (i.e., tunicamycin, A23187, E-64). At the end of the incubation period, the cells were thoroughly rinsed in DPBS and incubated for an additional 2–3 h in normal DMEM-5% FCS (i.e., “recovery” period).

ATP depletion. After the so-called 2–3 h recovery period, the pretreated cells were rinsed twice in DPBS and then incubated for 0–6 h in the absence or presence of 10 µM antymycin A in DPBS supplemented with 1.5 mM CaCl₂ and 2 mM MgCl₂ to deplete cellular ATP (ATP-depleting conditions). Intracellular ATP concentrations were determined from separate dishes of cells using a modified luciferase assay (Sigma).

Northern blot analysis. Confluent monolayers of MDCK cells growing in 10-cm² tissue culture dishes were incubated for 0–16 h in DMEM-5% FCS with or without tunicamycin, A23187, or E-64. The cells were then rinsed twice in DPBS, scraped into a microfuge tube, and centrifuged at 4°C for 5 min at 3,000 rpm. The supernatant was removed, and total RNA was then isolated from the cell pellet by phenol-chloroform extraction. In the case of Northern blot analysis following ATP depletion, pretreated and nonpretreated cells were subjected to 2 h of ATP depletion followed by 4 h of ATP repletion in normal DMEM, after which total RNA was isolated. The RNA was electrophoresed on 1% formamide-agarose gels and transferred to nitrocellulose as previously described (3, 17). The nitrocellulose blots were hybridized with random primed [³²P]cDNA for either BiP or Hsp70 overnight at 42°C, washed with saline-sodium citrate and exposed to autoradiographic film.

Trypan blue exclusion assay. After preinduction of ER chaperones and 6 h of ATP depletion as previously described, confluent monolayers of MDCK cells growing in 12-well tissue culture dishes were carefully rinsed twice in PBS and exposed for 5 min to 0.1% trypan blue in PBS supplemented with 1.5 mM CaCl₂ and 2 mM MgCl₂. The cells were examined using an inverted Nikon Labphot microscope, and the number of viable and nonviable cells was determined.

LDH assay. Confluent monolayers of MDCK cells growing in 96-well titer plates were incubated with or without ER chaperone-inducing agents for 12–16 h and subjected to ATP depletion for 6 h as described. A total of 200 µl of ATP-depleting medium was added to each well. To compare total cellular LDH with LDH released into the medium due to cell death, 10% Triton X-100 was added to final concentration of 0.1% to one-half of the wells before LDH determination. The plates were incubated at 1,000 rpm for 10 min at 4°C, and 100 µl of supernatant were removed from each well to a separate 96-well titer plate. To each well, 100 µl of LDH-colorimetric determination solution (Boehringer-Mannheim) were added. The plates were incubated in subdued light for 10 min at room temperature. Determination of LDH content was done using an inverted Nikon Diaphot microscope, and the amount of released LDH to total cellular LDH was calculated.

Fluorescent cell death assay. MDCK cells growing on glass coverslips were incubated for 12–16 h with the various agents and subjected to 6 h of ATP depletion as previously described. The cells were rinsed in DPBS and incubated for 45 min at room temperature in the Live/Dead fluorescent dyes (Molecular Probes) diluted in PBS supplemented with 1.5 mM CaCl₂ and 2 mM MgCl₂. Calcein-acetoxymethyl ester, a dye indicating viable cells, is transported across the plasma membrane in the form of a cleavable ester. The cleavage of the ester occurs only in viable cells and activates the fluorescence at a wavelength comparable to that of FITC. Ethidium homodimer, which indicates nonviable cells, enters cells in which the integrity of the plasma membrane is lost and binds to nuclear DNA. The DNA binding activates the fluorescent dye at a wavelength comparable to that of tetramethylrhodamine B isothiocyanate. Determination of viable versus nonviable cells was done using a Nikon Labphot microscope equipped with epifluorescence. The ratio of nonviable cells versus the total number of cells was determined.

Cycloheximide treatment. Confluent monolayers of MDCK cells growing in 96-well titer plates were rinsed in DPBS and incubated for 12–14 h in the absence or presence of 1 µM tunicamycin and 50 µM cycloheximide. After this pretreatment period, the cells were rinsed in DPBS and allowed to recover in fresh DMEM-5% FCS for 2–3 h prior to depletion of ATP with antimycin A as previously described. Cellular viability (i.e., plasma membrane integrity) was measured using the LDH release assay.

RESULTS AND DISCUSSION

Over the past several years, a number of studies have demonstrated that overexpression of cytosolic Hsps can protect cells and tissues subjected to ischemia or ATP depletion. For example, heat shock treatment and overexpression of Hsps have been correlated with increased cellular survival following ischemia and reperfusion in whole tissues, including heart, retina, neurons, brain, and kidney (1, 7, 16, 21, 25, 27, 31, 32). Furthermore, preinduction of Hsp70 by mild heat shock protects cultured cells from injury due to ATP depletion by antymycin A (9, 15, 37). In addition, preinduction of both Hsp70 and ER molecular chaperones with inhibitors of the cytosolic proteasome protects MDCK cells from thermal injury (2). Although it is unclear how the overexpression of Hsps protects cells from damage, the enhanced viability is thought to be due, at least in part, to an increase in the “chaperoning” capacity of the cells. Thus, overexpression of Hsps (presumably protects the cells by providing more chaperones involved in the renaturation, refolding, or degradation of denatured, misfolded, or aggregated proteins, which accumulate during ischemia and reperfusion, and other kinds of injury (2, 6, 12, 14, 32, 35, 38).

We have recently shown that whole kidney ischemia or ATP depletion of cultured renal and thyroid epithelial cells induces ER molecular chaperones (i.e., an ER stress response), in addition to a cytosolic stress response (17). In cultured cells, messages coding for the ER molecular chaperones can also be increased by treatment with agents that cause the accumulation of unfolded proteins in the ER (i.e., an ER stress response) (2, 5, 18, 20, 28). For example, treatment of animal cells with tunicamycin, a nucleoside antibiotic that blocks N-linked glycosylation of secretory and transmembrane proteins in the ER, can induce the transcription of several ER molecular chaperones including BiP, grp94, ERP72, and FKBP13 (3, 17). Thus, as expected, treatment of MDCK cells for 12–16 h with 0.1–10 µM tunicamycin elicited an ER stress response as indicated...
by the pronounced rise in the levels of mRNA encoding BiP (Fig. 1), one of many ER chaperones induced under these conditions. Because a concomitant induction of the cytosolic Hsps was not observed, we wondered if this agent might prove useful in evaluating the potential cytoprotective properties of ER chaperones in the ATP-depletion model for ischemic injury, particularly as a preliminary study had already raised this possibility (40).

Consequently, mitochondrial oxidative phosphorylation was inhibited in MDCK cells (both control and pretreated) with antimycin A (17, 35). Treatment of MDCK cells with 10 µM antimycin A caused a rapid decrease in the intracellular level of ATP in both control cells and those that had been treated for 12–16 h with tunicamycin (Fig. 2). Despite the fact that the intracellular levels of ATP for both control and tunicamycin-pretreated groups were essentially zero for the majority of the experiment, the initial rates of decline in intracellular ATP levels were somewhat slower in tunicamycin-pretreated cells (Fig. 2). However, by 60 min the levels for both groups were ≤5% of control levels, and by 120 min the levels of ATP for both groups were below the limit of detection (Fig. 2). These intracellular levels of ATP remained at this low level for the entire time that the cells were exposed to antimycin A (i.e., up to the 6-h time point when cell injury was measured). Because it has been shown that cellular injury and stress response are dependent on the level and duration of ATP depletion (36), it is possible that the differences in the initial intracellular ATP decline between the two groups could have had an effect on the viability of the cells. However, the finding that intracellular ATP levels of ~50% were found to be the threshold for the initiation of a stress response (36), together with the fact that the ATP level for each treatment group was essentially zero for more than 5 h, the relatively minor difference in the rate of decline would argue against such a possibility. Thus it seems reasonable to conclude that pretreatment with tunicamycin did not cause the cells to retain high levels of ATP in the face of long-term antimycin A treatment and that the observed cytoprotection is due to its other effect(s), presumably on the ER.

Northern blot analysis demonstrated that treatment of MDCK cells with 10 µM antimycin A followed by recovery for 4 h caused both an ER and a cytosolic stress response as indicated by the marked increases in the mRNA encoding both Hsp70 and BiP (Fig. 3). Interestingly, ATP depletion of tunicamycin-pretreated MDCK cells led to additional increases in the mRNA level of BiP (compared with cells not pretreated with tunicamycin), but not Hsp70 mRNAs (compare lanes 2 and 4, Fig. 3). This suggests that the capacity of the cells to induce ER molecular chaperones is not maximized by 12–16 h of treatment with tunicamycin and that the two stresses (tunicamycin and ATP depletion) have additive effects on the levels of this ER molecular chaperone. These results also confirm that tunicamycin treatment does not elicit a cytosolic stress response (i.e., induction of the Hsps), nor does pretreatment interfere with the cell’s ability to mount such a response in the face of ATP depletion.

Fig. 1. Tunicamycin treatment causes selective increases in levels of mRNA encoding the endoplasmic reticulum (ER) molecular chaperone BiP. Northern blot analysis of total RNA collected from Madin-Darby canine kidney (MDCK) cells grown in DMEM-5% FCS for 12–16 h in the absence (control, lanes 1 and 7) or presence of various concentrations of tunicamycin (lane 2, 10 µM; lane 3, 1 µM; lane 4, 0.1 µM; lane 5, 10 nM; lane 6, 1 nM). Blot was probed with [32P]cDNA probes for gene products indicated at left. Ethidium bromide staining of 28s RNA was used to indicate that equal amounts of RNA were loaded on the gels. This figure is representative of at least 3 separate experiments.

Fig. 2. Treatment with antimycin A depletes cellular ATP levels. Graph showing effects of treatment of MDCK cells with 10 µM antimycin A on the intracellular level of ATP in control and tunicamycin-pretreated cells.
To determine if preinduction of ER molecular chaperones was associated with an effect on cell damage, MDCK cells (control and tunicamycin pretreated) were subjected to ATP depletion and the extent of cellular injury (i.e., plasma membrane integrity) to the cells was determined and compared. Using trypan blue exclusion, a well-characterized and much utilized measure of cell viability (4), we found a significant decrease in the level of cell injury (≈80%) induced by ATP depletion in MDCK cells pretreated with tunicamycin (Fig. 4), suggesting that ER molecular chaperones preinduced by tunicamycin might play a cytoprotective role in cells subjected to ATP depletion. This phenomenon was investigated further by examining the ability of other agents known to induce ER molecular chaperones (such as the calcium ionophore A23187) to affect MDCK cell injury following ATP depletion.

As with tunicamycin, treatment of MDCK cells for 12–16 h with A23187 selectively caused an ER stress response as indicated by the increased transcription of BiP, but not cytosolic Hsp70 (Fig. 5, lane 3). In contrast, the cysteine protease inhibitor E-64, which enters cells and has been reported to interfere with the degradation of some proteins in the ER (39), did not cause increases in the mRNA levels of either ER (BiP) or cytosolic (Hsp70) chaperones (Fig. 5, lane 4), and was used as a negative control in the experiments. Based on the ability of A23187 to selectively induce the ER molecular chaperones, we investigated its potential cytoprotective effects in ATP-depleted MDCK cells. However, because calcium ionophores also disrupt intracellular calcium homeostasis, thereby potentially complicating interpretation, the subsequent experiments were performed in tandem with tunicamycin.

Two different measures of cell injury were employed: LDH release and a fluorescent dye-based assay (see Experimental Procedures). Cellular LDH is only released from cells when the plasma membrane is damaged, ultimately leading to cell death. Thus LDH release (detectable in the growth medium) is a sensitive measure of cell viability (i.e., plasma membrane damage) (4), and increases in LDH in the culture medium are indicative of the presence of dead and/or dying cells. Preinduction of ER molecular chaperones with tunicamycin or A23187 significantly reduced LDH release from ATP-depleted MDCK cells (Fig. 6). For example, pretreatment with tunicamycin or A23187 caused a reduction in LDH release approaching 60%, indicating a significant reduction in the level of cell injury after ATP depletion (Fig. 6). In contrast, E-64 did not induce...
ER chaperones or alter the level of LDH release (Fig. 6), suggesting that induction of an ER stress response (i.e., induction of ER molecular chaperones) is necessary for the observed cytoprotective effect.

A fluorescent assay for cell viability provided additional support for this hypothesis. Microscopic examination of MDCK cells grown on coverslips showed a marked decrease in the number of nonviable cells in tunicamycin-pretreated cells (compare Fig. 7, C and D). Treatment of MDCK cells with tunicamycin reduced the level of cell injury by almost 80%, compared with uninduced controls (Fig. 7E). This reduction in plasma membrane damage following tunicamycin pretreatment was comparable to that seen using the trypan blue exclusion assay (compare Figs. 4 and 7E). A23187 also reduced cell death, although not to the same level as tunicamycin pretreatment (Fig. 7E). However, whereas the calcium ionophore reduced cell injury by ~60%, E-64 appeared to accelerate cell injury in this assay (Fig. 7E).

Thus all three independent measures of cell injury suggest that agents that cause a selective induction of ER molecular chaperones protect cells from damage due to ATP depletion. To strengthen the temporal association between ER-chaperone induction and cytoprotection, MDCK cells were pretreated with tunicamycin for varying time periods (3–14 h) prior to being subjected to ATP depletion. The results demonstrate that as little as 3 h of treatment with tunicamycin, which coincides with the point at which the first demonstrable evidence for BiP mRNA induction is noted (Fig. 8A, lane 3), also coincides with the onset of cytoprotection in the face of ATP depletion (Fig. 8B). Despite increasing levels of BIP induction with time, near maximal protection is achieved at the 3-h time point. This may represent a threshold phenomenon where a marginal increase from the constitutive level of BIP production is adequate to be cytoprotective or may imply that BIP transcription does not adequately reflect that of the other potential chaperones involved. In addition, because ATP depletion was performed following a 2- to 3-h recovery period following treatment with either tunicamycin or A23187, it is possible that this is a sufficient period of time to “rev-up” the level of ER chaperones to a cytoprotective level. Thus the lack of a robust increase of BiP mRNA on the Northern blot (which was performed immediately following the treatment with either tunicamycin or A23187) might not be truly indicative of the amount of chaperone protein at the time of ATP depletion. Nevertheless, the possibility that other nonchaperone-dependent mechanisms of cytoprotection are also activated by treatment with agents that induce ER molecular chaperones cannot be completely excluded.

As stated above, the data demonstrate a correlation between ER chaperone induction and cytoprotection. However, they do not prove that the induction of ER chaperones per se directly leads to cytoprotection. For example, A23187 has other cellular effects (i.e., perturbation of cellular calcium homeostasis) and the possibility that the decreases in cell injury were due to something other than overexpression of ER molecular chaperones remained. Although this seems less likely in the case of tunicamycin, believed to be a specific

\[ \text{ER CHAPERONES AND ENHANCED CELL SURVIVAL} \]

**Fig. 6.** Pretreatment with agents that elicit an ER stress response decrease cell damage caused by ATP depletion. Graph of MDCK cell death as measured by lactate dehydrogenase (LDH) release assay following 6 h of ATP depletion in medium containing 10 µM antimycin A. Cells were pretreated in absence (control) or presence of either 1 µM tunicamycin, 1 µM A23187, or 100 mM E-64. Data are expressed as means ± SE; n = 8. *P < 0.001.

**Fig. 7.** Inducers of ER molecular chaperones increase cell survival following ATP depletion. A–D: fluorescent analysis of MDCK cells grown for 12–16 h in absence (control, A and C) or presence of either 1 µM tunicamycin (B and D) followed by 6 h of incubation in ATP-depletion medium. A and B: calcein-acetoxymethyl ester fluorescent analysis of living cells. C and D: ethidium homodimer analysis of dead cells. E: graph of MDCK cell death as measured by fluorescent Live/Dead (Molecular Probes) kit after 6 h of ATP depletion. Cells were pretreated in the absence (control) or presence of either 1 µM tunicamycin, 1 µM A23187, or 100 mM E-64. Data are expressed as means ± SE; n = 3. *P < 0.05. **P < 0.001.
that the concentration of cycloheximide used was inadequate to completely abolish new protein synthesis. Furthermore, as discussed below, it is also possible that BiP induction does not accurately reflect the contribution of other ER chaperones to cytoprotection. Nevertheless, although the data suggest that multiple mechanisms may be involved in the acquisition of cytoprotection, it is likely that ER molecular chaperones play a major role.

Although the data presented suggest that ER chaperone synthesis mediates at least a component of the observed cytoprotection, especially given the high specificity of tunicamycin, direct implication of the ER chaperones may require using other protocols to overexpress the individual chaperones such as BiP (e.g., transgenic mice or transfected cells) or necessitate using antisense technology to block their synthesis, as has been done for various Hsps (22, 34). It is important to emphasize that tunicamycin and A23187 induce a variety of ER luminal proteins (BiP, grp94, ERp72, and FKBP13) with chaperone function and that it is likely that multiple ER chaperones participate in the folding of membrane and secreted proteins (2, 3, 5, 17–20, 28). Given their potential overlapping specificities and structural homologies, it therefore seems quite plausible that affecting the expression of a single chaperone may not sufficiently affect the folding environment of the ER (especially in the face of concomitant high expression of other chaperones) to significantly alter cell survival as measured here. Nevertheless, it will be important to pursue some of these approaches, primarily to try to characterize the individual contribution of each ER chaperone (i.e., BiP, grp94, ERp72, calreticulin, protein disulfide isomerase, etc.) to the observed cytoprotective effect against ATP depletion.

The data presented here provide evidence that pretreating cells with agents that induce ER molecular chaperones results in cytoprotection in the face of ATP depletion. This is, at least in part, a protein-mediated effect, and, much like the case of cytosolic Hsps, the precise mechanism of protection remains to be elucidated. One may speculate that aside from the likely

![Figure 8: Time course of ER chaperone induction correlates with cytoprotection. A: Northern blot analysis of total RNA collected from MDCK cells grown in DMEM-5% FCS for 12–16 h in the absence (control, lane 1) or presence of 1 µM tunicamycin for 1 h (lane 2), 3 h (lane 3), 6 h (lane 4), and 14 h (lane 5), respectively. The blot was probed with a [32P]cDNA probe for BiP, and ethidium bromide staining of 28S rRNA was used to indicate the amounts of RNA that were loaded on the gels. This figure is representative of 2 experiments. B: graph of MDCK cell death as measured by LDH release assay after 6 h of ATP depletion. Cells were treated as previously described before being ATP depleted and assayed for LDH release. Data are expressed as means ± SE; n = 8. P < 0.004 for all groups vs. control.](http://ajprenal.physiology.org/)

![Figure 9: Cycloheximide-induced protein translational arrest attenuates tunicamycin-mediated cytoprotection. Graph of MDCK cell death as measured by LDH release after 6 h of ATP depletion. MDCK cells were pretreated in the absence (1, solid bar, control, no tunicamycin or cycloheximide; hatched bar, 50 µM cycloheximide) or presence of 1 µM tunicamycin (2, open bar, 1 µM tunicamycin alone without cycloheximide; hatched bar, 1 µM tunicamycin and 50 µM cycloheximide) for 12–14 h. Data are expressed as means ± SE; n = 24. P < 0.001 compared with control.](http://ajprenal.physiology.org/)
positive effect on the folding environment, increased levels of chaperones ameliorate severe cellular stress by binding potentially toxic or malformed proteins within the ER, which could potentially be toxic to the cell. Another mechanistic possibility centers on the demonstrated ability of ER chaperones to function as calcium-binding proteins (23, 29, 30). As the ER also serves as a major intracellular reservoir for this cation, which has been implicated in epithelial cell injury, it is possible that increased levels of ER chaperones serve as a “sink” for otherwise toxic calcium concentrations that develop following ATP depletion and thereby protect the cells from further injury. In support of this hypothesis, recent studies have shown that ER chaperones play an important role in enabling the cell to resist oxidative damage by buffering the subsequent rise in intracellular calcium (24). This feature may also be instrumental in stabilizing the ER calcium pool, preventing apoptosis from being triggered (13) and thereby bestowing the cell with a marked survival benefit.

Given our postulate, what physiological role might ER molecular chaperones play in the setting of ischemic injury and recovery? Epithelial cells establish and maintain their characteristic phenotype, at least in part, through the action of a number of specialized cellular structures and processes (i.e., adherens and tight junctions, and cell-cell substrate attachments). ATP depletion or ischemia has been shown to disrupt these structures and processes, leading to loss of the permeability barrier and apical-basolateral polarity, as well as impaired cell-substrate interactions (8, 11, 26). Because the folding and assembly of the integral membrane and secreted proteins that comprise these structures occur in the ER and are mediated by ER molecular chaperones, the ability to recover from ischemic insults would be expected to depend on the ability of the cell to replace or repair these proteins. Thus preinduction of the ER molecular chaperones could enhance cell survival by increasing the chaperoning capacity of the ER, for example, for junctional proteins, cell adhesion molecules, and integrins, thereby increasing the rate at which that recovery of cell function occurs. Strategies to induce ER chaperones could conceivably be useful in clinical settings, either as preemptive measures or to enhance recovery after injury.

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