Upregulation of the SERCA-type Ca2+ pump activity in response to endoplasmic reticulum stress in PC12 cells

ABSTRACT

These findings provide the first functional evidence for the increase of SERCA pumping capacity in cells subjected to the ER stress. Since at least three different and unrelated mechanisms of eliciting the ER stress response were found to cause this functional upregulation of Ca2+ transport into the ER, these results support the existence of a coupling between the induction of the UPR pathway in general, and the regulation of expression of at least one of the SERCA pump isoforms.

INTRODUCTION

Background Endoplasmic reticulum (ER) is a compartment simultaneously involved in the processes of protein synthesis and Ca2+ homeostasis in eukaryotic cells. Nascent polypeptide chains entering the secretory pathway, as well as extracytosolic portions of proteins destined for the external or internal cell membranes, translocate into the ER lumen. Within the ER, posttranslational processes including folding, glycosylation, subunit assembly and transfer to the Golgi compartment take place, assisted by chaperone proteins residing in the ER lumen. In addition, proper protein folding and maturation depends on the maintenance of the oxidative milieu and high Ca2+ concentrations within the ER. Reducing agents, depletion of ER lumenal Ca2+, inhibition of glycosylation or interference with the secretory pathway (by preventing transfer to the Golgi system), each lead to an accumulation of misfolded protein intermediates and increase the demand on the chaperoning capacity. Such conditions, collectively termed ER stress, have been shown to induce ER-specific stress response pathways. Two ER stress pathways concerned with protein processing are recognized today. The Unfolded Protein Response (UPR) was originally described in yeast, and has been more recently demonstrated in mammalian cells. In mammals, the proximal ER stress-sensing element of this pathway appears to be Ire1p, a 110 kDa, highly conserved protein spanning the ER membrane. It is thought that when misfolded polypeptide chains accumulate within the ER causing a decrease in the level of free chaperones, the lumenal N-terminal portion of Ire1p undergoes dimerization. This in turn leads to a trans-autophosphorylation of the Ire1 cytosolic domains, triggering an ER-to-Nucleus (ERN) signaling. The details of the ERN signaling are not understood at present. It appears to involve additional activities, in part residing in the cytosolic portion of the Ire1p molecule itself (RNA endonuclease activity), and in part contributed by proteins recruited through binding to Ire1p cytosolic domain (e.g. TRAF2,). Overall, the major result of the UPR induction is a transcriptional upregulation of a number of stress proteins, including members of the family of Glucose Regulated Proteins (GRPs) such as GRP78 (BiP) and GRP94 (endoplasmin), as well as other chaperones (calreticulin, protein disulfide isomerase, ERp72), thereby responding to the increased demand for the folding capacity within the ER. Another ER-specific stress response has been termed Endoplasmic Reticulum Overload Response (EOR)), and is triggered by certain of the same conditions known to activate UPR (e.g. glucose deprivation, glycosylation inhibition), as well as by heavy overexpression of proteins within the ER. The distinguishing feature of EOR is its association with the activation of the transcription factor NF-κB. While necessary for the folding and maturation processes within the ER, the high concentration of Ca2+ ions in this compartment (1000-10,1000 times higher than in the cytosol) is also a prerequisite for the universally

employed Ca2+ signaling through ER Ca2+ channels (inositol 1,4,5-trisphosphate and ryanodine receptors). This high ER Ca2+ concentration is maintained by ER Ca2+ pumps, members of the family of Sarco-Endoplasmic Reticulum Calcium ATPases (SERCAs). Depletion of ER Ca2+ has been shown to cause a transcriptional induction of the mRNA for SERCA pumps. However, the upregulation of an actual SERCA activity in such circumstances has not been demonstrated. In the present work, we provide the first functional evidence that depletion of ER Ca2+ during cell culture leads to an increase in the SERCA pumping capacity, as expressed by an enhancement of SERCA-mediated 45Ca2+ uptake to microsomes isolated from Ca2+ -depleted PC12 cells. In addition, we show that similar functional SERCA upregulation may also be elicited by conditions of ER stress previously shown not to be accompanied by Ca2+ depletion. Taken together with our earlier results, the present data support the existence of a linkage between the induction of UPR and the regulation of SERCA gene expression.

CONCLUSION

Conclusions This work shows for the first time an upregulation of ER Ca2+ -ATPase (SERCA) activity upon a disturbance of the internal ER environment by several agents known to evoke the ER stress response, or UPR. Since UPR may be activated by a variety of unrelated stimuli, e.g. Ca2+ depletion, inhibition of glycosylation, misfolding due to faulty amino acid precursors, energy deprivation or interference with the ER oxidative milieu, it may be thought of as a "final common pathway" for signaling ER stress caused by a range of factors. Therefore, we suggest that the UPR pathway is a likely candidate for the common mechanism to underlie the upregulation of SERCA activity following treatments with agents as different in their modes of action as EGTA, tunicamycin and DTT. Within this proposed common mechanism for SERCA activation due to UPR, some variation in the response amplitude between the individual agents appears to exist (Fig. 2). Further work will be required to understand the level (transcriptional and/or translational), and the exact mechanisms of such differences. We have recently reported that treatment of PC12 cells with EGTA, tunicamycin, DTT or brefeldin A induced approximately 3- to 4-fold increases in the mRNA for SERCA2b, and approximately 2-fold increases in SERCA2b immunoreactive protein, with no corresponding changes for SERCA3. An upregulation of SERCA activity was also supported in this earlier work by an increase in the amount of SERCA phosphoenzyme. However, we would like to stress that despite these indications, the actual demonstration by the present paper of the stress-induced increase in the capacity of Ca2+transport into the ER was important for at least 2 reasons. First, as previously discussed [, p.22371], technical difficulties precluded an accurate comparison of the extent of ATPase upregulation as measured at the immunoprotein and the phosphoenzyme level. Second, we were concerned with the possibility that ER stress, even if causing an increased synthesis of the immunoreactive protein, might at the same time by its very nature result in a faulty posttranslational processing of SERCA2b, with a degree of functional impairment. Since, as shown by the mutagenesis work, it is possible to disrupt the Ca2+ transport cycle of Ca2+ -ATPase without affecting at least some of the partial phosphorylation reactions, an actual full measurement in the present paper of the thapsigargin-sensitive Ca2+ transport into the ER was necessary to unequivocally demonstrate the validity of the ER stress-induced upregulation of SERCA2b activity.