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Expression and induction of the stress protein alpha-B-crystallin in vascular endothelial cells

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Abstract Stress-induced development of enhanced tolerance against various kinds of stresses has been observed in vascular endothelial cells as well as in several other cell types. Stress proteins are thought to play a key role in the development of stress tolerance. In this study we show that endothelial cells of various sources contain the major stress protein of the eye lens, αB-crystallin. In the mouse myocardial microvascular cell line, MyEnd, αB-crystallin as well as the heat shock proteins HSP 70i and HSP 25 display a low constitutive expression but can be significantly upregulated by sodium arsenite stress. Osmotic stress also resulted in strong upregulation of αB-crystallin and HSP 70i but not of HSP 25. Both osmotic and arsenite stress resulted in significant stress tolerance of MyEnd cells against glucose deprivation as assayed by lactate dehydrogenase release and overall cellular morphology. Development of stress tolerance without induction of HSP 25 indicates that HSP 25 is not essential for the protective effect. MyEnd cells from αB-crystallin-/- mice displayed a similar degree of stress tolerance showing that αB-crystallin is dispensable for protection of cells against energy depletion. The functional role of αB-crystallin in endothelial cells needs to be further elucidated. In our experiments HSP 70i turned out to be the only potential candidate of the stress proteins assayed to be involved in the development of tolerance against energy depletion.

Keywords αB-Crystallin · Endothelium · Stress proteins · Stress tolerance

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Introduction

The most fundamental cellular defense mechanism against stress in a wide range of different cell types is the so-called heat shock response which implies the expression of members of the highly conserved group of heat shock proteins (HSPs). αB-Crystallin, primarily identified as a major protein of the eye lens, is one member of to the family of small HSPs (Ingolia and Craig 1982). αB-Crystallin is not only expressed in the lens but occurs also in several non-lenticular tissues and cell types such as skeletal muscle, cardiomyocytes, glial cells, and fibroblasts (Bhat and Nagineni 1989; Dubin et al. 1989; Iwaki et al. 1990). Like other HSPs αB-crystallin has been demonstrated to possess chaperone-like activities that prevent different proteins such as alcohol dehydrogenase in vitro from denaturation and aggregation (Horwitz 1992). In striated muscle αB-crystallin is constitutively highly expressed and has been shown to rapidly translocate from a cytoplasmic pool to myofibrillar components upon stress conditions (Chiesi et al. 1990; Barbato et al. 1996; Golenhofen et al. 1998, 1999). In contrast, in non-muscle cells such as glial cells and fibroblasts αB-crystallin displays a constitutive low expression and responds to various kinds of stresses with upregulation of synthesis (Klemenz et al. 1991; Inaguma et al. 1992) and association with intermediate filaments (Djabali et al. 1997). Binding of αB-crystallin to intermediate filament proteins (desmin, vimentin, glial fibrillary acidic protein) and modulation of intermediate filament organization has been shown in vitro (Bennardini et al. 1992; Nicholl and Quinlan 1994; Perng et al. 1999). The precise function of αB-crystallin is still unknown, but its stress-induced association with the cytoskeleton indicates that it may contribute to stress tolerance by some kind of protection of the cellular scaffold. αB-Crystallin could exert such a protective effect by preventing cytoskeletal components from stress-induced denaturation, depolymerization, and aggregation.

In vascular endothelial cells αB-crystallin has so far not been identified and there is also little information

about the expression of other HSPs in the endothelium. However, vascular endothelial cells are known to be capable of stress-induced responses that improve their survival against a variety of adverse conditions such as hyperthermia (Ketis et al. 1988), hypoxia/ischemia (Loktionova and Kabakov 1998; Loktionova et al. 1998), and oxidative stress (Gill et al. 1998). Protection against ischemic injury was suggested to involve stabilization of the actin filament cytoskeleton by HSP 27 (equivalent to rodent HSP 25). HSP 27/25 belongs together with αB-crystallin to the group of small HSPs. Analysis of human endothelial cells by 2D-gelelectrophoresis demonstrated the presence of several HSPs including HSP 27, HSP 60, HSP 70c, and HSP 70i (Portig et al. 1996). Thus, endothelial cells appear to be equipped with a large set of HSPs the function of which in endothelial pathobiology remains to be elucidated.

In the present study we focused on αB-crystallin which displays negligible constitutive expression but responds with pronounced upregulation upon osmotic stress and arsenite treatment. However, upregulation of αB-crystallin is not essential for arsenite and osmotic shock induced tolerance against glucose depletion (main determinant of hypoxia/ischemia).

Materials and methods

Electrophoresis and immunoblotting

Endothelial cells were dissolved in Laemmli sample buffer (Laemmli 1970) and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7.5% and 13% gels). Protein contents were quantified according to Heinzel et al. (1965). For immunoblotting, proteins were transferred in Kyhse-Andersen transfer buffer (Kyhse-Andersen 1984) to Hybond nitrocellulose membranes (Amersham, Braunschweig, Germany) which were blocked with 5% low fat milk in phosphate-buffered saline (PBS; pH 7.4) for 3 h at RT and incubated overnight at 4°C with the respective primary antibody (in PBS plus 5% low fat milk). A polyclonal rabbit antibody against bovine lenticular αB-crystallin was prepared and affinity purified as described (Golenhofen et al. 1998). Another polyclonal rabbit antibody against αB-crystallin was obtained from Stressgen (Victoria, Canada). Both antibodies were used at a dilution of 1:2,000–1:3,000. Monoclonal antibodies against the following HSPs (HSP 25, 60, 70c, 70i, and 90) were obtained from Stressgen and used at a dilution of 1:1,000. The affinity-purified phospho-specific antibodies to αB-crystallin were a kind gift of R.A. Quinlan (dilution 1:500). The polyclonal rabbit antibody against human vimentin has been characterized previously and used at a dilution of 1:1,500 (Drenckhahn and Wagner 1986). As secondary antibody horseradish peroxidase-labeled goat anti-rabbit or anti-mouse IgG (Jackson Immunoresearch Laboratories, West Grove, Pa., USA) was used diluted 1:3,000 with PBS. Bound immunoglobulins were visualized by the enhanced chemiluminescence technique (ECL; Amersham).

Isolation of endothelial cells for immunoblotting

Porcine thoracic aortae and inferior venae cavae were obtained from the local slaughterhouse. Vessels were cut longitudinally, spread, and fixed on a cork pad with pins. After rinsing with PBS (4°C) and removal of small blood clots, the endothelial layer was covered with PBS (4°C) containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 µg/ml leupeptin, aprotinin, and pepstatin) and cells were then removed by gentle scraping with a rubber policeman. The resulting cell suspension was centrifuged for 10 min at 400 g. The cell pellet was dissolved in Laemmli sample buffer and processed for immunoblotting as described above.

Cultured porcine arterial endothelial cells (PAECs), human umbilical cord vein endothelial cells (HUVECs), and mouse myocardial endothelial cells (MyEnd)

Pulmonary PAECs and HUVECs were harvested and characterized as described previously (Suttorp et al. 1988; Schnittler et al. 1993). Cells were cultured in medium 199 containing 10% fetal calf serum (Gibco, Karlsruhe, Germany) or 20% human serum (obtained from the local blood bank) for HUVECs, passaged once a week, and used for experiments between passages 2 and 5.

Immortalized cell lines of endothelial cells from myocardium [MyEnd, MyEnd (αB-/-)] were generated from wildtype and αB-crystallin-deficient mice that also lack the myotony-kinase-binding protein (MKBP also called HSPB2; Wawrousek and Brady 1998). MKBP could not be demonstrated by Western blotting to be expressed in MyEnd cells neither in control nor in stress conditions indicating that MKBP is not relevant for endothelial function. Cell lines were generated by transformation with Polyoma virus middle T antigen (PymT) as described previously for microvascular endothelial cells from brain (Aumailley et al. 1991). In brief, myocardial tissue of newborn mice was minced, digested with 0.05% trypsin (Biochrom, Berlin, Germany) and 0.02% collagenase (Boehringer, Mannheim, Germany), and seeded onto gelatine-coated culture dishes. Cells were grown in Dulbecco's modified Eagle medium (Life Technologies, Eggenstein, Germany) containing 10% fetal calf serum (Biochrom) and 50 U/ml penicillin/streptomycin. One day after cell isolation adherent cells (mainly fibroblasts and endothelial cells) were transfected with PymT using the retrovirus packaging cell line GP+E-86 (Markowitz et al. 1988) that was kindly provided by B. Engelhardt (Bad Nauheim, Germany). PymT transfection causes growth advantage of endothelial cells over non-endothelial cells leading to a homogenous monolayer of cells with endothelial morphology after 4–6 weeks of culture. MyEnd and MyEnd (αB-/-) cells were immunopositive for several endothelial marker proteins, such as von Willebrand factor, VE-cadherin, and PE-CAM-1. Cultures were used for experiments between passages 5 and 20.

Stress exposure

MyEnd cells were grown on gelatine-coated tissue culture dishes or flasks. Exposure to heat shock was achieved by incubating cells grown in culture flasks for 30 or 60 min in a waterbath at 42°C. In some experiments 50 µM nordihydroguaiaretic acid (NDGA; Sigma, Deisenhofen, Germany) was added to the culture medium during the heat shock period as well as 15 min before and thereafter as described (Ito et al. 1996). As a further kind of stress 100 µM sodium arsenite was added to the cultures for 2 h and allowed to recover for 16 h. In some experiments cells were exposed subsequently to a second time period of 2 h to 100 µM sodium arsenite. Osmotic stress was achieved by culturing cells for 24 h with a culture medium containing an excess of 100 and 200 mM NaCl which corresponds to an increase of osmolarity from 300 mosmol/l (control) to 500 and 700 mosmol/l, respectively. Cells were allowed to recover for 24 h in control medium before being analyzed.

Subcellular fractionation

MyEnd cells were scraped off, lysed, and homogenized in 20 mM TRIS (pH 7.4) containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 µg/ml leupeptin, aprotinin, and pepstatin).

The homogenate was centrifuged at 40,000 *g* for 30 min and the supernatant centrifuged again at 150,000 *g* for 3 h. Both pellets and the 150,000 *g* supernatant were dissolved in equal volumes of Laemmli sample buffer and subjected to immunoblotting.

Lens extracts and purified αB-crystallin

Mouse eye lenses were homogenized in PBS and subsequently centrifuged for 1 h at 100,000 *g*. The resulting supernatant was dissolved in Laemmli sample buffer and used for immunoblotting. Purification of αB-crystallin was performed from bovine eye lenses as previously described (Golenhofen et al. 1998). Purification included gel filtration (Sepharyl S-300) and subsequent anion exchange chromatography (DEAE-cellulose).

Stress tolerance

MyEnd cells pretreated with sodium arsenite or osmotic stress as described above as well as untreated cells were exposed to experimental energy depletion. Energy depletion was performed by incubating cells with 10 mM deoxyglucose in physiological salt solution (115 mM NaCl, 4.7 mM KCl, 25 mM $NaHCO_3$, 1.2 mM $MgCl_2$, 1.2 mM KH_2PO_4, 1.5 mM $CaCl_2$). Controls were performed by replacing deoxyglucose with glucose. At time points 8, 24, and 48 h, samples of the culture supernatant were taken and assayed for lactate dehydrogenase (LDH) activity. LDH activity was determined by the rate at which pyruvate was reduced which is coupled with the oxidation of NADH and can be followed spectrophotometrically by reduction of the absorbance at 340 nm (lactate dehydrogenase kit; Sigma). In addition, cells were examined for viability by microscopic inspection of cell morphology. Data are presented as means ± SEM of four to seven experiments and tested for significance by the Student's *t*-test ($P<0.05$).

Results

Detection of αB-crystallin in the vascular endothelium

Figure 1 shows Western blots for αB-crystallin of native endothelial cells freshly isolated from pig thoracic aorta and inferior caval vein as well as of cultured endothelial cells from porcine pulmonary trunk (PAECs) and human umbilical cord vein (HUVECs). Endothelial cells of all sources displayed an immunoreactive band at 21 kDa with the same electrophoretic mobility as purified lenticular αB-crystallin demonstrating endothelial cells as a further cell type expressing αB-crystallin.

Induction of αB-crystallin

For studying the amount of endothelial αB-crystallin at stress conditions the murine myocardial microvascular endothelial cell line MyEnd was chosen. The MyEnd (αB-/-) cell line served as control. MyEnd cells displayed a very low constitutive αB-crystallin content which was hardly detectable by the Western blotting conditions applied (Fig. 2 *lane 2*). MyEnd cells exposed to heat shock (30 and 60 min at 42°C) and subsequent recovery at 37°C for 16 h displayed no (30 min) or low level (60 min) of αB-crystallin induction (Fig. 2). Addition of 50 µM NDGA, a specific inhibitor of leukotriene synthesis, during the heat shock period, however, caused

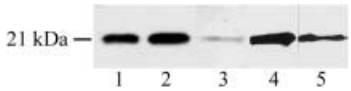

Fig. 1 Western blot analysis for αB-crystallin of purified αB-crystallin (*1*), cultured porcine arterial endothelial cells (*2*), cultured human umbilical cord vein endothelial cells (*3*), and freshly isolated endothelial cells from pig thoracic aorta (*4*) and inferior caval vein (5). Note abundance of αB-crystallin in all endothelial cells investigated

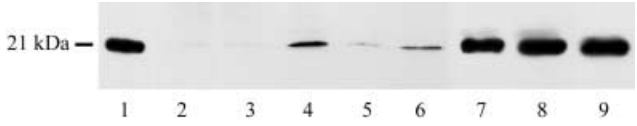

Fig. 2 Western blot analysis for αB-crystallin of MyEnd cells after different kinds of stress exposure. αB-Crystallin displays low constitutive expression (*lane 2*) and is hardly induced upon heat shock (*lane 3* 30 min 42°C, *lane 5* 60 min 42°C). Combination of heat shock and 50 µM nordihydroguaiaretic acid (NDGA) led to a slight increase in αB-crystallin synthesis (*lane 4* 30 min 42°C plus NDGA, *lane 6* 60 min 42°C plus NDGA). Strongest induction of αB-crystallin was observed by exposure to osmotic stress (*lanes 7, 8* excess of 100 mM and 200 mM NaCl) and to 100 µM sodium arsenite (*lane 9*). *Lane 1* Purified αB-crystallin as a standard

significant increase of αB-crystallin amount. The strongest induction of αB-crystallin was observed by either exposure to 100 µM sodium arsenite (2 h of exposure plus 16 h recovery) or osmotic stress at an excess of 100 and 200 mM NaCl (24 h stress exposure plus 24 h recovery). MyEnd (αB-/-) cells exposed to these stress conditions did not express αB-crystallin. No induction of αB-crystallin synthesis in MyEnd cells could be shown by the following conditions known to stimulate expression of αB-crystallin in fibroblasts or glial cells: 0.1–5 mM H_2O_2, 10–100 µM *N*-acetyl-sphingosine, 0.5–5 µg/ml anisomycin, and 1–50 µM dexamethasone (not shown).

Subcellular compartment, state of phosphorylation of αB-crystallin

In various cell types αB-crystallin has been reported to translocate in response to stress conditions to cytoskeletal structures, especially to the intermediate filament system (see Introduction). This process is often accompanied by phosphorylation of αB-crystallin (see Introduction). To investigate if αB-crystallin is associated in MyEnd cells with vimentin, the predominating intermediate filament protein of endothelial cells (Franke et al. 1979; Schnittler et al. 1998), cells were fractionated by centrifugation and analyzed for αB-crystallin and vimentin by Western blotting. MyEnd cells were pretreated with 100 µM sodium arsenite for 2 h (followed by 16 h recovery) to induce αB-crystallin synthesis as described above. Since binding of αB-crystallin to cytoskeletal structures may occur only during the stress period itself in some experiments a second stress period of 2 h 100 µM sodium arsenite was performed. Figure 3A, B shows that after both stress condi-

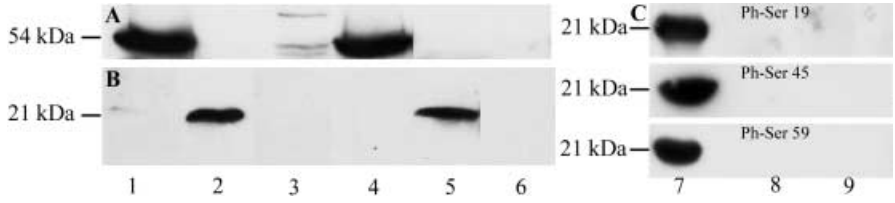

Fig. 3 Western blot analysis of MyEnd cells for vimentin (**A**) and αB-crystallin (**B**) after single exposure to 100 µM arsenite (*1, 2, 3*) and double exposure to arsenite (*4, 5, 6*). Homogenates of cells were fractionated by centrifugation in a 40,000 *g* pellet (*1, 4*), 150,000 *g* pellet (*2, 5*), and 150,000 *g* supernatant (*3, 6*). Whereas vimentin was restricted to the 40,000 *g* pellets (*1, 4*), αB-crystallin was found in the 150,000 *g* pellets (*2, 5*). **C** Western blot analysis with phospo-specific antibodies to αB-crystallin of mouse lens homogenate (*7*), MyEnd cells treated for one period with arsenite (*8*), and MyEnd cells stressed a second time with arsenite (*9*). Whereas all antibodies directed against phospho-Ser 19, 45, and 59 of αB-crystallin reacted with mouse lens homogenates no phosphorylation of αB-crystallin in MyEnd cells could be detected

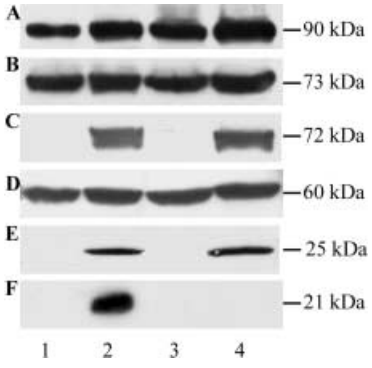

Fig. 4 Western blot analysis for HSP 90 (**A**), HSP 70c (**B**), HSP 70i (**C**), HSP 60 (**D**), HSP 25 (**E**), and αB-crystallin (**F**) of untreated MyEnd cells (*1*), MyEnd cells treated with arsenite (*2*), untreated MyEnd (αB-/-) cells (*3*), and MyEnd (αB-/-) cells treated with arsenite (*4*). Note constitutive expression of HSP 90, 70c, and 60 and induction of HSP 70i, HSP 25, and also to a lesser degree of HSP 90 in both cell lines. αB-crystallin was induced only in MyEnd cells and, as expected, not in MyEnd (αB-/-) cells

tions vimentin was found in the 40,000 *g* pellet whereas αB-crystallin remained in the supernatant and sedimented only at 150,000 *g*. Thus, endothelial αB-crystallin (upregulated by stress) is not associated with vimentin filaments. In addition, samples of these cell lysates were investigated for phosphorylation by antibodies specific for the three known phosphorylation sites of αB-crystallin, i.e., Ser 19, Ser 45, and Ser 59. Whereas all three antibodies reacted with extracts of the mouse lens known to contain phosphorylated αB-crystallin no reaction with MyEnd cells could be observed (Fig. 3C).

Other stress proteins

The endothelial cell line generated from myocardium of αB-crystallin-deficient mice [MyEnd (αB-/-)] was virtually indistinguishable from MyEnd cells with respect to growth properties, morphology, and expression of several endothelial marker proteins (PECAM-1, VE-cadherin, von Willebrand factor). Both cell lines were compared in terms of expression of other stress proteins under constitutive as well as stress conditions (2 h sodium arsenite, 16 h lag time). Figure 4 shows Western blots for αB-crystallin, HSP 25, HSP 60, HSP 70i, HSP 70c, and HSP 90. Lack of αB-crystallin in MyEnd (αB-/-) is demonstrated in Fig. 4F. HSP 60, 70c, and 90 were constitutively expressed in both MyEnd and MyEnd (αB-/-) cells. MyEnd (αB-/-) cells did not show any compensatory upregulation of the stress proteins assayed. In contrast to mouse myocardium the small heat shock proteins HSP 20 and MKBP were not detectable in both cell lines, neither under control nor stress conditions (data not shown). Arsenite exposure caused strong induction of HSP 25 and HSP 70i and moderate increase of HSP 90. Exposure of both cell lines to osmotic stress (excess of 100/200 mM NaCl) caused induction of HSP 70i and moderate induction of HSP 90 but not of HSP 25 (Fig. 5).

Stress tolerance in MyEnd cells

Short sublethal periods of stress have been shown in various cell types to account for enhanced tolerance to a second stress period such as ischemia/hypoxia. MyEnd and MyEnd (αB-/-) cells were investigated for resistance to energy depletion prior to and after induction of stress proteins with sodium arsenite and osmotic stress. Energy depletion was performed by incubation of cell cultures with 10 mM deoxyglucose in physiological salt solution. At time points 8, 24, and 48 h cells were examined for injury by determination of LDH activity in samples of the cell culture supernatant (Fig. 6). Moreover overall cellular morphology was determined by phase contrast microsco-

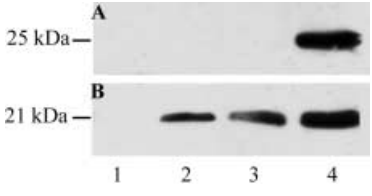

Fig. 5 Western blot analysis of HSP 25 (**A**) and αB-crystallin (**B**) of MyEnd cells (*1*), MyEnd cells treated with osmotic stress, i.e., excess of 100 mM NaCl (*2*) and of 200 mM NaCl (*3*), and MyEnd cells exposed to arsenite (*4*). Note induction of αB-crystallin by arsenite as well as osmotic stress whereas HSP 25 was only induced by arsenite and not by osmotic stress

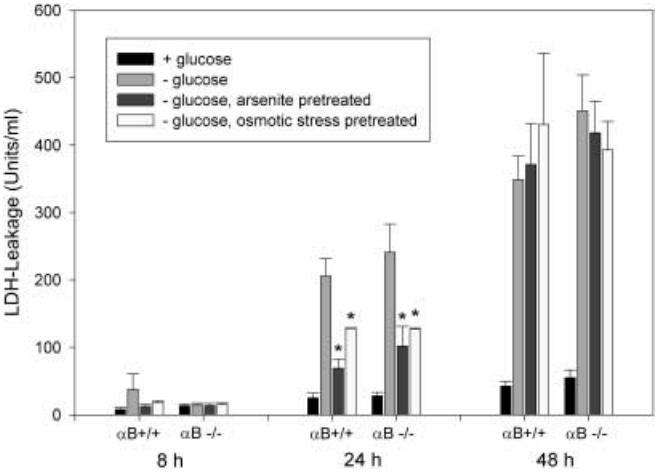

Fig. 6 Lactate dehydrogenase (LDH) leakage of cultures of MyEnd and MyEnd (αB-/-) cells after energy depletion by glucose deprivation. Twenty-four hours after energy depletion a strong increase in LDH release of MyEnd and MyEnd (αB-/-) cells was observed compared to control cultures plus glucose. Cultures pretreated with arsenite as well as osmotic stress demonstrated significantly lower LDH release (*asterisks* significantly different from cultures without pretreatment, $P<0.05$)

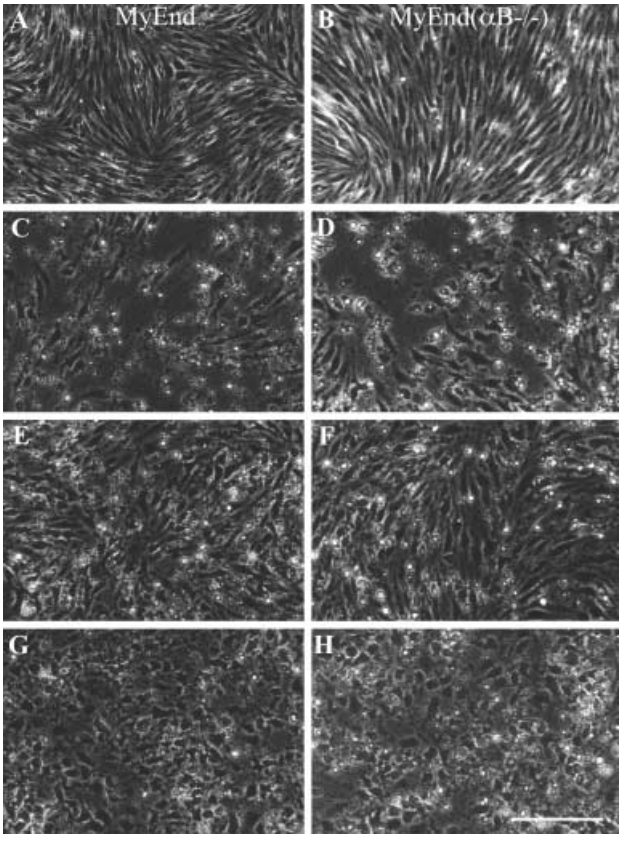

Fig. 7 Cultures of MyEnd (**A**, **C**, **E**, **G**) and MyEnd (αB-/-) cells (**B**, **D**, **F**, **H**) at control conditions (**A**, **B**) and after 24 h of energy depletion (**C**–**H**). Note preserved cell morphology of cultures pretreated with arsenite (**E**, **F**) or osmotic stress (**G**, **H**) compared to cultures without pretreatment (**C**, **D**). *Bar* 100 µm

py (Fig. 7). During 8 h of energy depletion no significant changes could be observed between cultures with and without prior stress exposure. LDH leakage was as low as in control cells cultured with glucose containing physiological salt solution. However, cell cultures exposed to energy depletion for 24 h displayed severe damage. Monolayers lost confluency and LDH activity in the supernatant increased from control values of 25±7 LD units/ml (MyEnd) and 28±5 LD units/ml [MyEnd (αB-/-)] to 206±27 LD units/ml (MyEnd) and 241±42 LD units/ml [MyEnd (αB-/-)]. Differences between MyEnd and MyEnd(αB-/-) cells were not significant. Pretreatment with arsenite and osmotic stress led to a statistically significant lower LDH release in response to energy depletion [arsenite pretreatment: 69±13 LD units/ml in MyEnd and 102±29 LD units/ml in MyEnd (αB-/-); osmotic stress pretreatment: 128±1 LD units/ml in MyEnd and 127±2 LD units/ml in MyEnd (αB-/-)]. Reduced LDH leakage seen after energy depletion in cells pre-exposed to arsenite or osmotic stress correlated to overall cellular structure. Unstressed cultures displayed considerable loss and rounding up of cells 24 h after energy depletion whereas cell cultures pre-exposed to stress showed less severe cellular changes (Fig. 7).

Discussion

αB-Crystallin in endothelial cells

αB-Crystallin has been shown to be expressed not only in the eye lens but also in striated muscle, fibroblasts, glial cells, and some epithelial cells (Bhat and Nagineni 1989; Dubin et al. 1989; Iwaki et al. 1990). In this study we show that αB-crystallin is also expressed in vascular endothelial cells. αB-Crystallin could be detected in both native and cultured cells. However, constitutive expression of αB-crystallin in endothelial cells is rather low as compared to cardiomyocytes where αB-crystallin comprises up to 2% of the soluble cellular protein content. In MyEnd cells the amount of αB-crystallin was strikingly upregulated in response to certain stress conditions (osmotic stress, arsenite pretreatment) which have been also shown in cultured glial cells to induce αB-crystallin upregulation (Head et al. 1994; Ito et al. 1995). However, MyEnd cells differ from glial cells and fibroblasts in that heat shock even in combination with NDGA had only a weak effect on upregulation of αB-crystallin (Inaguma et al. 1992; Klemenz et al. 1993; Tumminia and Russell 1994; Ito et al. 1996). Moreover, in MyEnd cells αB-crystallin was not upregulated by treatment with dexamethasone, H_2O_2, *N*-acetyl-sphingosine or anisomycin, conditions that have been shown to cause induction of αB-crystallin in glial cells or fibroblasts (Aoyama et al. 1993; Chang et al. 1995). Differences in the responsiveness to certain stress conditions between endothelial cells and glial cells/fibroblasts suggest the existence of alternate cell type-specific stress-induced signaling pathways. This makes biological sense because different

types of cells exposed in situ to different environmental stress conditions may require different stress-mediated signaling pathways to protect them against cell type- or tissue-specific forms of stresses.

With respect to the association of αB-crystallin with the cytoskeleton endothelial cells differ from other cell types. Whereas in fibroblasts, glial cells and also cardiomyocytes αB-crystallin translocates during various stress conditions from cytosol to cytoskeletal components (Chiesi et al. 1990; Djabali et al. 1997; Golenhofen et al. 1998; Perng et al. 1999) we could not see in MyEnd cells any redistribution of αB-crystallin to the cytoskeleton-containing 40,000 *g* pellet in response to either arsenite or osmotic stress. In addition, immunostaining of MyEnd cells exposed to arsenite and osmotic stress revealed diffuse cytosolic αB-crystallin distribution with no preferential localization to actin filament stress fibers, intermediate filaments, or microtubules (data not shown). One way to explain this discrepancy may be our finding of the absence of stress-induced phosphorylation of αB-crystallin in MyEnd cells. In other non-endothelial cells phosphorylation of αB-crystallin is a hallmark of stress-induced translocation of αB-crystallin to the cytoskeleton (Djabali et al. 1997; Golenhofen et al. 1998). The cellular targets of endothelial αB-crystallin still remain to be shown.

Stress tolerance in the myocardial endothelium

Hearts exposed to sublethal stresses such as short periods of ischemia or heat shock display improved myocardial survival after a second prolonged ischemic period, a phenomenon known as ischemic preconditioning (Murry et al. 1986; Currie et al. 1988; Marber et al. 1993). Preconditioning is not limited to myocytes but also delays ischemic dysfunction of coronary endothelial cells (Richard et al. 1994; Bouchard and Lamontagne 1996; Pohlman and Harlan 2000; Rubino and Yellon 2000). In this study we could confirm that cultured endothelial cells are also capable of developing tolerance to ischemia-like conditions (energy depletion). Tolerance to energy depletion was achieved by pretreatment with short periods of arsenite or hypertonic stress. Since αB-crystallin, HSP 25, HSP 70i, and to a lesser extent HSP 90 were found to be induced upon arsenite pretreatment these stress proteins are possible candidates for mediating this cytoprotective effect. However, HSP 25 (the rodent homologue of human HSP 27) which has been suggested earlier to induce protection against ischemic or oxidative injury in endothelial cells (Loktionova and Kabakov 1998; Loktionova et al. 1998), was not induced by hypertonic treatment in our cell system and thus is not essential for the protective effect. αB-Crystallin has been shown to be responsible for increased survival of cardiomyocytes during ischemic injury (Martin et al. 1997; Ray et al. 2001) but does not seem to play a major role in development of stress tolerance in MyEnd cells under the experimental conditions investigated. MyEnd (αB-/-) cells were protected to a similar degree as MyEnd cells and did not show compensatory upregulation of other heat shock proteins. Among the three inducible stress proteins examined, i.e., HSP 25, HSP 70i, and αB-crystallin only HSP 70i was upregulated in MyEnd (αB-/-) cells in response to osmotic stress indicating HSP 70i as a potentially important stress protein of ischemic protection of endothelial cells. This conclusion is supported by studies on cardiomyocytes which demonstrated that overexpression of HSP 70i is of protective value during ischemic injury (Marber et al. 1995; Plumier et al. 1995). The functional implication of upregulation of the small heat shock proteins HSP 25 and αB-crystallin in MyEnd cells remains unclear. Both proteins may contribute to the observed cytoprotective effect to a minor extent or may play a protective role during other stresses not tested in this study.

In conclusion, αB-crystallin is expressed at low levels in vascular endothelial cells and becomes significantly upregulated by arsenite and osmotic stress but not by heat shock. However, its function in endothelial cells remains to be determined. No differences between MyEnd and MyEnd (αB-/-) cells in development of stress tolerance could be observed.

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