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Protein phosphatase inhibitors and heat preconditioning prevent Hsp27 dephosphorylation, F-actin disruption and deterioration of morphology in ATP-depleted endothelial cells

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Abstract The vascular endothelium response to ischemic depletion of ATP was studied in vitro. Endothelial cells (EC) cultured from human aorta or umbilical vein were incubated in a glucose-free medium containing CCCP or rotenone. Such blockade of energy metabolism caused a drop in ATP, destruction of actin filaments, morphological changes, and eventually disintegration of EC monolayer within 2–2.5 h. While ATP fell and F-actin collapsed, the 27-kDa heat shock protein (Hsp27) lost basal phosphorylation and became Triton X-100-insoluble forming granules inside the cell nuclei. Protein phosphatase (PP) inhibitors (okadaic acid, cantharidin, sodium orthovanadate) did not delay the ATP decrease in energy-deprived EC but arrested both the alterations in the Hsp27 status and the changes for the worse in F-actin and cell morphology. Similarly, the Hsp27 dephosphorylation/insolubilization/granulation and the cytoskeletal and morphological disturbances resulting from lack of ATP were suppressed in heat-preconditioned (thermotolerant) cultures, this effect being sensitive to quercetin, a blocker of Hsp induction. The longer preservation of the cytosolic pool of phosphorylated Hsp27 during ATP depletion in the PP inhibitor-treated or thermotolerant EC correlated with the acquired resistance of F-actin and morphology. These data suggest that PP inhibitors as well as heat-inducible Hsp(s) can protect ischemia-stressed cells by preventing the ATP loss-provoked protein dephosphorylation and breakdown of the actin cytoskeleton.

Key words: Heat shock protein; Actin; Protein phosphatase; Ischemia; Vascular endothelial cell

1. Introduction

A severe fall of cellular ATP is one of the most detrimental consequences of ischemia or anoxia that deranges the cytoskeleton and its interactions with the plasma membrane, thus dramatically affecting cell morphology and viability (see for review [1,2]). Organization of microfilaments and their contacts with the plasma membrane are mainly regulated by protein kinases and protein phosphatases (PP) via phosphorylation/dephosphorylation of actin-associated and regulatory proteins [1,3]. Depletion of ATP in cells during ischemia or anoxia may shift the balance between phosphorylation and dephosphorylation toward the latter, since all kinase reactions require ATP. Indeed, anoxia in rabbit proximal renal tubules completely suppressed protein phosphorylation, whereas protein dephosphorylation was unchanged or enhanced [4]. Such abnormal prevalence of protein dephosphorylation over phosphorylation was suggested as a probable cause of the cytoskeletal collapse under ischemia or anoxia [1,4–6]. In support of this suggestion, dephosphorylation of two actin-regulating proteins, ezrin in anoxic rabbit proximal renal tubules [5] and the 27-kDa heat shock protein (Hsp27) in ATP-depleted human endothelial cells (EC) [6], was shown to occur and, in both cases, this was accompanied by disorganization of microfilaments.

At the same time, an increase in intracellular Hsp(s) appears to allow ATP-deprived cells to longer preserve intact F-actin, normal morphology and viability [2,7–10]. Despite the obvious importance of this phenomenon nothing is known about its molecular basis. It was discussed that direct Hsp-actin binding may protect microfilaments from fragmentation and aggregation during ATP depletion [2,11] but there is no evidence in favor of such a mechanism. Now we speculate about an ability of excess Hsp(s) somehow to attenuate the protein dephosphorylation in ATP depleted cells that is critical for the state of the actin cytoskeleton. To examine this we have focused our studies on Hsp27. The main in vivo functions of this protein are actin reorganization in response to cell-activating stimuli [12,13] and protection of F-actin under stresses [14–18]. In cells overexpressing Hsp27, the actin networks were more resistant to heating [14,15], oxidants [16,17] and cytochalasin D [14,18] that was associated with improved survival of the treated cells. The protective potential of human Hsp27 toward microfilaments is up-regulated by phosphorylation of its serine residues Ser^{15}, Ser^{78} and Ser^{82} in a stress- and mitogen-sensitive signaling pathway [15–18]. In contrast, non-phosphorylated Hsp27 is able to inhibit actin polymerization [12,13,19]. Moreover, phosphorylation/dephosphorylation of Hsp27 can alter its oligomeric structure, solubility and localization in cells thereby influencing its functional (actin-regulating) activity [6,15,19,20]. Thus, if one explores how the ATP loss-enhanced protein dephosphorylation affects the microfilament stability, Hsp27 seems to be a suitable object for monitoring.

In our last report [6], we showed that sustained ATP depletion in human vascular EC reduces basal phosphorylation of Hsp27; simultaneously, Hsp27 becomes Triton-insoluble and forms granules inside the cell nuclei. Under such conditions, endothelial microfilaments break and aggregate that results in loss of normal morphology and barrier properties of EC monolayer [21–24]. As we discussed above, the F-actin dis-

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Abbreviations: Hsp, heat shock protein; EC, endothelial cells; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; PP, protein phosphatase; PBS, phosphate buffered saline; TRITC, tetramethylrhodamine isothiocyanate; IEF, isoelectric focusing; ECL, enhanced chemiluminescence

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ruption in ATP-deprived cells may be connected with the stress-provoked dephosphorylation of the microfilament constituents and accessory proteins (e.g. ezrin, Hsp27 and others). To prevent protein dephosphorylation under ATP depletion we used PP inhibitors sodium orthovanadate, okadaic acid and cantharidin, two latter being known to block Hsp27 dephosphorylation in vivo [20,25]. In parallel, we elevated the Hsp content in EC by heat preconditioning; herein we looked for development of the cytoskeletal tolerance to lack of ATP as it was demonstrated for other cells [7–10]. The main goal of the present work was to reveal the expected relation between the Hsp27 phosphorylation, the F-actin stability, and the level of Hsp expression in the context of ATP depletion.

2. Materials and methods

2.1. Cells, ATP depletion and ATP determination

EC were isolated from human aortas or umbilical veins and cultured according to described technique [26]. The confluent cell cultures of the 1–3 passages were taken for the experiments.

The cellular ATP stores were depleted by incubating the cells in glucose-free Dulbecco's modified Eagle's minimal essential medium (Sigma) containing 3% fetal bovine serum and supplemented with 20 μM carbonyl cyanide *m*-chlorophenylhydrazone (CCCP, a mitochondrial uncoupler) or 20 μM rotenone (a respiratory chain inhibitor) [6]. Cellular ATP was extracted and then measured by luciferin/luciferase method using ATP assay kit (Calbiochem) [6,8].

2.2. Tolerance-inducing treatments

To block in vivo protein phosphatase activity 1 μM okadaic acid (Calbiochem) or 0.2 μM cantharidin (Sigma), or 100 μM sodium orthovanadate (Aldrich) were added immediately in the incubation medium for all period of the ATP-depleting stress.

Thermotolerance developed after heating EC cultures in a thermostatic water bath at 45°C for 10 min followed by recovery at 37°C during 14–18 h. Cytoresistance to elevated temperature and to ATP depletion was tested using fluorescence and phase-contrast microscopy. Stability of the F-actin structures and preservation of normal EC morphology during 30 min heating (45°C) or 2 h ATP depletion were considered characteristic signs of the acquired tolerance.

2.3. Cell fractionation with Triton X-100

Growing in plastic 35 mm dishes confluent EC were lysed with 0.3 ml of ice-cold phosphate buffered saline (PBS, pH 7.4) containing 1% (w/v) Triton X-100, 3 mM ethylenediaminetetraacetic acid and inhibitors of proteolysis (30 μg/ml of leupeptin and 1 mM phenylmethylsulfonyl fluoride). The cell lysates were promptly centrifuged at $12000 \times g$ for 10 min at 4°C, and the pellets were then washed with the lysing buffer and resedimented. The first supernatants (Triton-soluble cellular fraction) and the washed pellets (Triton-insoluble cellular fraction) were prepared as samples for Laemmli electrophoresis [6].

2.4. Fluorescence staining

EC adherent to gelatin-covered coverslips were fixed and permeabilized with a mixture of 3.7% formaldehyde/0.1% Triton X-100 for 10 min. Thereafter the preparations were washed trice with PBS and incubated with 1% bovine serum albumin for 30 min to decrease a non-specific staining. In situ F-actin and Hsp27 were labeled with phalloidin-TRITC conjugates (Sigma) and anti-Hsp27 rabbit antibodies (kindly provided by Dr. M. Gaestel [27]) as previously described [6]. The stained samples were viewed and photographed on an Opton III microscope (Karl Zeiss, Germany).

2.5. Electrophoresis, immunoblotting and isoelectric focusing

Total EC or cellular fractions were dissolved in a reducing sample buffer with the inhibitors of proteolysis, boiled for 5 min, and run by electrophoresis in Laemmli system with 4% stacking and 12.5% separating polyacrylamide gels. For Western blotting, separated proteins were electrotransferred from the gel slabs onto nitrocellulose membrane (0.45 μm, Bio-Rad). Hsp27 and inducible Hsp70 (Hsp70i) were detected using rabbit anti-Hsp27 antibodies [27] and monoclonal antibody C92F3A-5 specific for Hsp70i (StressGen) followed by labeling with anti-rabbit IgG and anti-mouse IgG-peroxidase conjugates, respectively. Images of the antigen bands developed on X-ray film (Amersham) by means of the enhanced chemiluminescence (ECL) method [6].

For isoelectric focusing (IEF), confluent EC in 60 mm dishes were lysed into 8 M urea supplemented with 5%, β-mercaptoethanol, 2% Nonidet P-40, and 100 μM sodium orthovanadate. Aliquots of the lysates were loaded in tubes with 5% polyacrylamide gel containing 8 M urea, 2% Nonidet P-40, and 2% ampholines (LKB, pH 5–7) and exposed to 500 V overnight and then to 1000 V for 1 h. Electrotransfer of proteins from the gel rods onto nitrocellulose was performed according to Zhou et al. [28]. HSP27 isoforms were detected by immunoblotting with anti-HSP27 antibodies and ECL as described above.

3. Results

3.1. PP inhibitors attenuate effects of ATP depletion on Hsp27, F-actin and EC morphology

Here we demonstrate how cell-permeant PP inhibitors ameliorate the EC responses to energy deprivation. The following reagents were tested: (i) sodium orthovanadate mainly

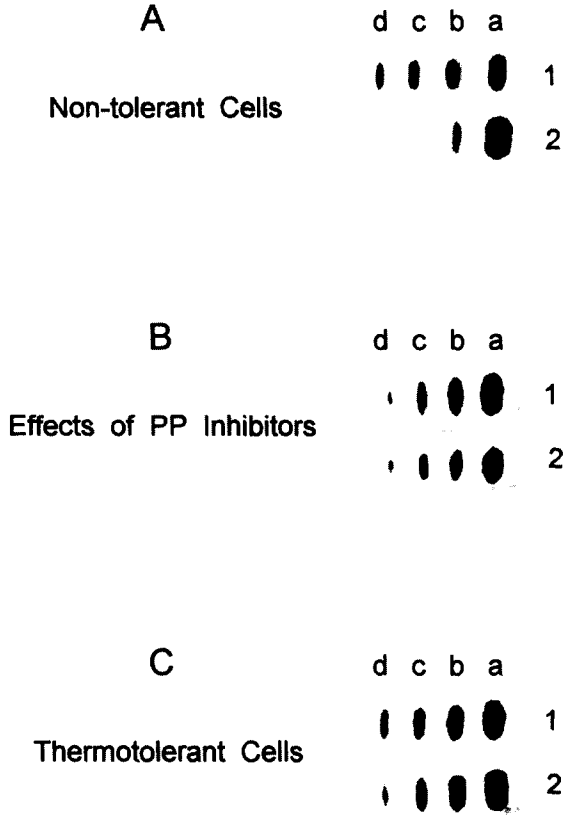

Fig. 1. PP inhibitors and acquired thermotolerance suppress Hsp27 dephosphorylation in ATP-depleted EC. Each horizontal row of spots represents a Hsp27 isoform spectrum in sample aliquots from equal numbers of cells (3×10^5) which were separated by IEF and blotted using ECL. A: Isoforms of Hsp27 in control (non-tolerant) cells without stress (1) and after ATP depletion as a result of 2 h incubation in glucose-free medium containing 20 μM CCCP (2). B: Inhibitory effects of 1 μM okadaic acid (1) and 0.2 μM cantharidin (2) on Hsp27 dephosphorylation in cells subjected to 2 h ATP depletion as in A. The same effect as with okadaic acid and cantharidin was achieved with 100 μM sodium orthovananadate (not presented). C: Hsp27 isoforms in thermotolerant (preheated) cells without stress (1) and after 2 h ATP-depleting exposure as in A (2).

blocking tyrosine PP, (ii) okadaic acid, which inhibits serine/threonine PP1 and PP2A with the stronger effect toward the latter, and (iii) cantharidin, a fairly specific blocker of PP2A in vivo [25]. None of these inhibitors retarded the ATP loss in cells treated with CCCP or rotenone (Kabakov and Gabai, unpublished data). At the same time, all the EC responses to ATP depletion described in [6] were markedly attenuated in the presence of 100 μM sodium orthovanadate or 1 μM okadaic acid, or 0.2 μM cantharidin (all the concentrations were chosen as non-toxic for human EC). Each of these treatments inhibited both the dephosphorylation/redistribution of Hsp27 and the F-actin collapse and the deterioration of morphology in ATP-deprived EC cultures (Figs. 1–4).

In vivo human Hsp27 can comprise up to four isoforms corresponding to non-phosphorylated (*a*), monophosphorylated (*b*), biphosphorylated (*c*), and triphosphorylated (*d*) Hsp27 (see Fig. 1 and [6,15]). 2 h ATP depletion dramatically decreased the share of all the three phospho-isoforms and augmented the non-phosphorylated *a* isoform (Fig. 1A), whereas the PP inhibitors suppressed this shift in the isoform spectrum (Fig. 1B) thus indicating inhibition of the Hsp27 dephosphorylation. Being mostly localized to Triton-soluble fraction of unstressed EC Hsp27 is insolubilized during ATP depletion (Fig. 2A and [6]). This ATP loss-induced Hsp27 insolubilization slowed down by sodium orthovanadate, cantharidin (both not shown) and okadaic acid (Fig. 2B). Without stress, immunofluorescent patterns of endothelial Hsp27 display diffuse distribution in the cytoplasm and the nucleus, and sometimes cytoplasmic aggregates; when ATP falls, the Hsp27-containing granules show up inside all the cell nuclei (see Fig. 3A,C and [6]). Okadaic acid (Fig. 3E) as well as two other inhibitors used (not shown) suppressed intranuclear granulation of Hsp27 in ATP-depleted EC.

The attenuation of the Hsp27 dephosphorylation/insolubilization/granulation in EC losing ATP in the presence of either sodium orthovanadate or cantharidin, or okadaic acid seems to correlate with the cytoresistance. Cultured EC contain many straight F-actin bundles (so called stress fibers) (Fig. 3B) which are disrupted after 2 h ATP depletion (Fig. 3D). Contrary to that, the preexisting stress fibers are preserved within ATP-depleted EC if the incubation medium contained sodium orthovanadate or cantharidin (both not shown), or okadaic acid (Fig. 3F). The cobblestone structure intrinsic to an intact EC monolayer (Fig. 4A) breaks following the cytoskeletal collapse in ATP-deprived EC and, after 2 h ATP depletion, detachment of the damaged cells begins (Fig. 4B). Under the same conditions, the PP inhibitor-treated EC exhibit good morphology and the cell detachment is absent (Fig. 4C). Apparently, the PP inhibitors contribute to stabilization of the actin framework upon blockade of energy metabolism thus enabling ATP-depleted EC somewhat to retain correct shape, intercellular contacts and monolayer integrity.

3.2. *Heat preconditioning confers EC tolerance to ATP depletion*

Mild heat shock (45°C, 10 min) in cultured EC with subsequent recovery at 37°C during 12–18 h induced Hsp accumulation (Fig. 5) and thermoresistance. The latter was manifested in the improved state of the cytoskeleton and the integrity of cell monolayer after severe heat shock (45°C, 30 min) as compared with non-tolerant EC (not shown). Simultaneously, the preheated cells became resistant to the sustained (2–2.5 h) ATP-depleting stress, although the rate of the ATP decrease in them was not slower than that in the non-preheated cells. Intact thermotolerant EC had F-actin patterns unaltered relative to non-tolerant EC (see Fig. 3B). Being devoid of ATP, the thermotolerant cells preserved both

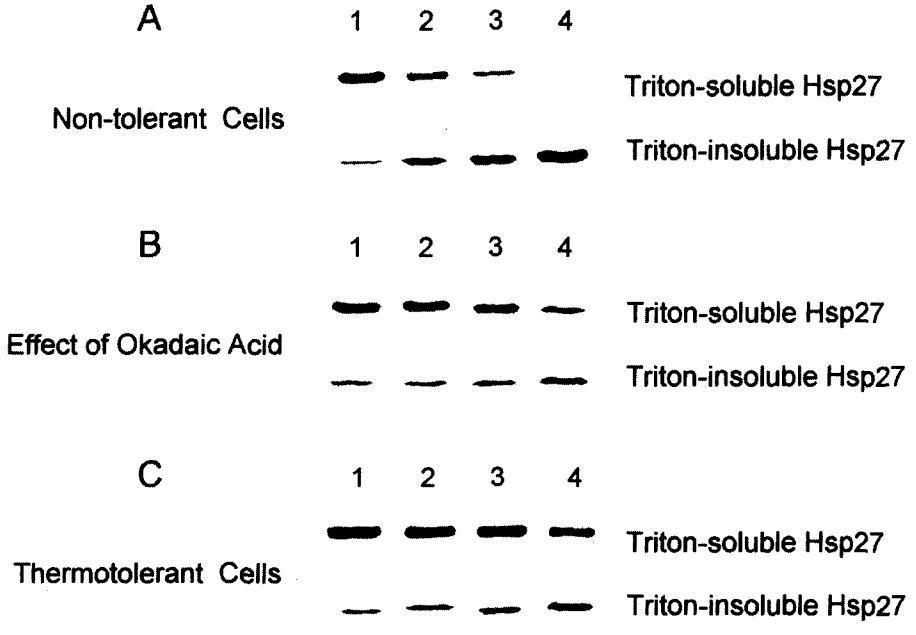

Fig. 2. PP inhibitors and acquired thermotolerance maintain soluble pool of Hsp27 in ATP-depleted EC. ECL blots show Hsp27 bands in sample aliquots of Triton X-100-soluble and -insoluble fractions obtained from equal numbers of cells (3×10^5). A: (1) unstressed non-tolerant cells; (2–4) 0.5 h, 1 h and 2 h incubation with 20 μM CCCP without glucose (ATP depletion). B: (1) unstressed cells; (2–4) 0.5 h, 1 h and 2 h ATP depletion as in A but in the presence of 1 μM okadaic acid. The same effects as with okadaic acid were achieved with 0.2 μM cantharidin and 100 μM sodium orthovananadate (both not presented). C: (1) unstressed thermotolerant cells; (2–4) time points of ATP-depleting exposure as in A.

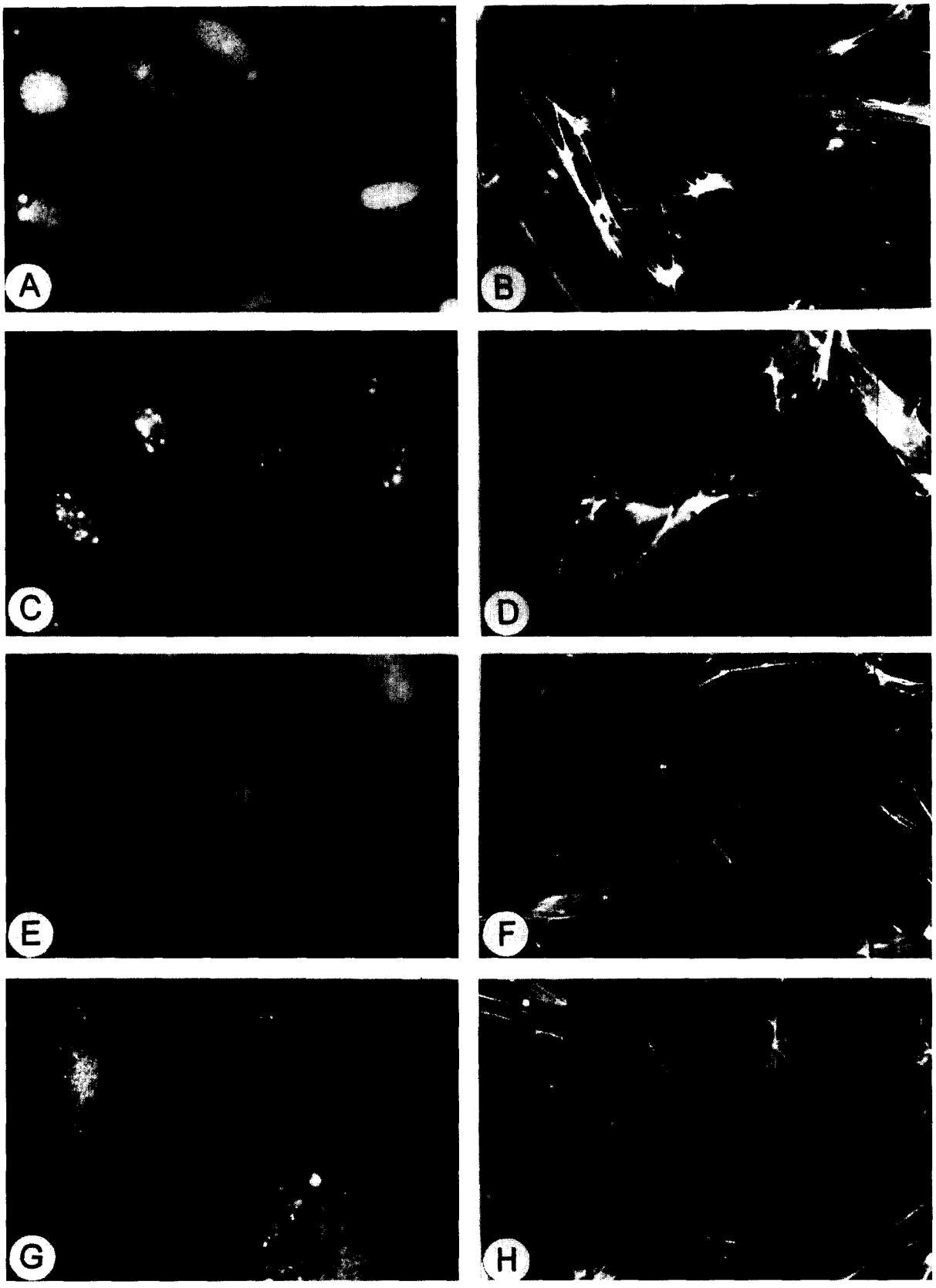

Fig. 3. Effects of PP inhibitors and acquired thermotolerance on distribution of Hsp27 and F-actin in ATP-depleted EC. A, B: Unstressed cells exhibiting usual distribution of Hsp27 (A) and intact F-actin fibers (B). C, D: Intranuclear granulation of Hsp27 (C) and F-actin disruption (D) after 2 h ATP depletion in non-tolerant cells. E, F: Prevention of intranuclear granulation of Hsp27 (E) and F-actin protection (F) during 2 h ATP depletion in the presence of 1 μM okadaic acid. The same results as with okadaic acid were observed with 0.2 μM cantharidin and 100 μM sodium orthovananadate (not presented). G, H: Suppression of intranuclear granulation of Hsp27 (G) and stability of F-actin fibers (H) after 2 h ATP depletion in thermotolerant cells. (Magnification ×400.)

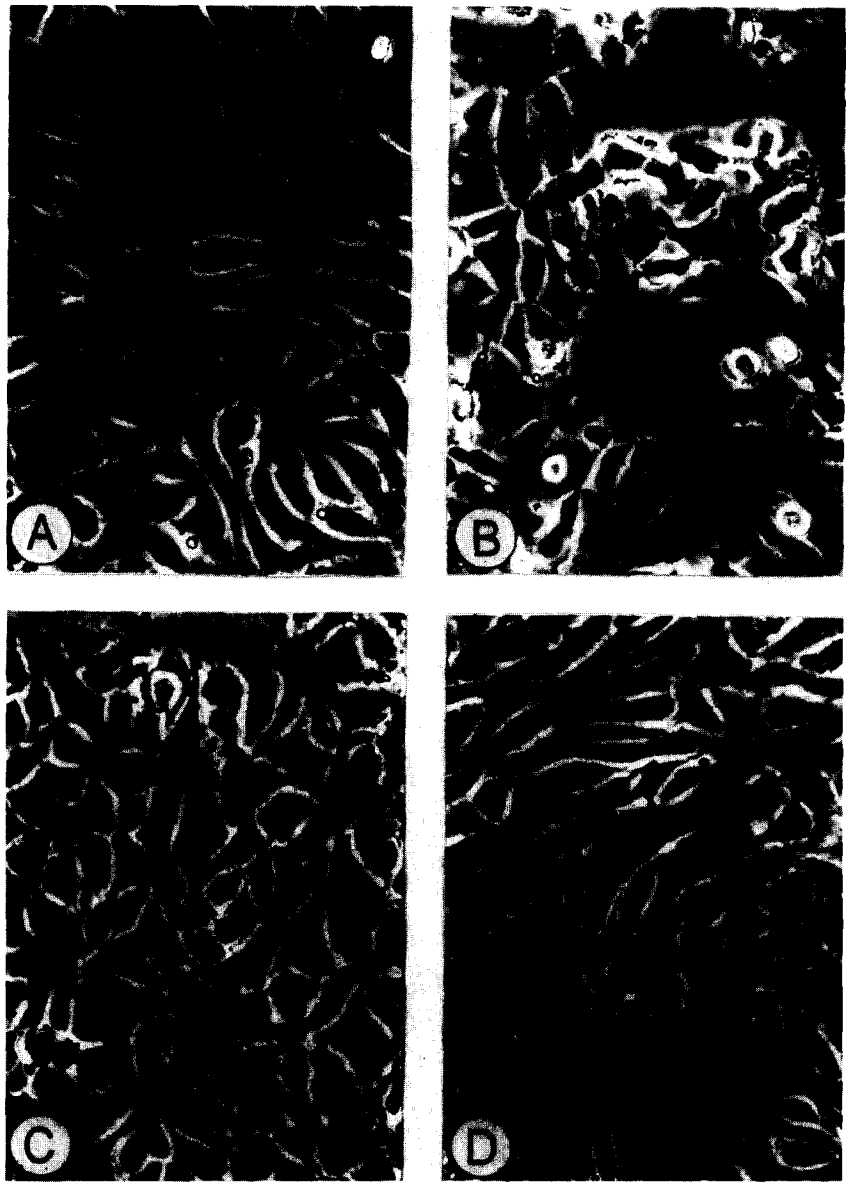

Fig. 4. Protective effects of PP inhibitors and acquired thermotolerance on morphology of ATP-depleted EC. A: Unstressed confluent culture exhibiting characteristic cobblestone structure. B: Deterioration of morphology and disintegration of monolayer resulting from 2 h ATP depletion (glucose deprivation+20 μM CCCP) in non-tolerant culture. C: Morphological tolerance of EC culture after 2 h ATP depletion as in B but in the presence of 1 μM okadaic acid. The same protective effects as with okadaic acid were observed with 0.2 μM cantharidin and 100 μM sodium orthovananadate (not presented). D: Improved preservation of morphology and integrity of cell monolayer in thermotolerant EC culture undergoing 2 h ATP depletion as in B. (Magnification ×100.)

the F-actin fibers (Fig. 3H) and normal morphology (Fig. 4D) for a long period (2–2.5 h). No cell detachment was found in the thermotolerant cultures after 3 h ATP deprivation. The acquired cytoresistance to ATP depletion finished in 25–30 h after heat shock; this was accompanied by loss of thermotolerance, decay of Hsp70i and diminution of the Hsp27 content to the initial level.

Like the PP inhibitors used, the heat shock-induced thermotolerance attenuated the Hsp27 reactions to depletion of ATP. Marked impairment of the ATP loss-provoked Hsp27 dephosphorylation was revealed in the samples of thermotolerant EC (Fig. 1C). Despite the increase in a total amount of endogenous Hsp27 after the heat pretreatment (Fig. 5), the Hsp27 insolubility during ATP depletion was less in thermotolerant EC than in the control cells (Fig. 2A,C). No Hsp27-containing granules were seen in nuclei of thermotolerant EC after 2 h ATP deprivation (Fig. 3G). Such an arrest of the Hsp27 dephosphorylation/insolubilization/granulation in ATP-depleted EC took place only in time limits of the acquired thermotolerance and correlated with the transient increase in Hsp expression. Thus, prior heat shock as well as the PP inhibitors provides a way to avoid the alterations in both Hsp27 and F-actin which result from ATP depletion. The microfilament resistance to lack of ATP appears to define the better preservation of the cell shape, cell-cell and cell-matrix contacts, and, as a consequence, the integrity of cell monolayer in thermotolerant EC cultures undergoing energy starvation.

To examine whether the effects observed in the thermotolerant cells depend on the Hsp accumulation, we added to

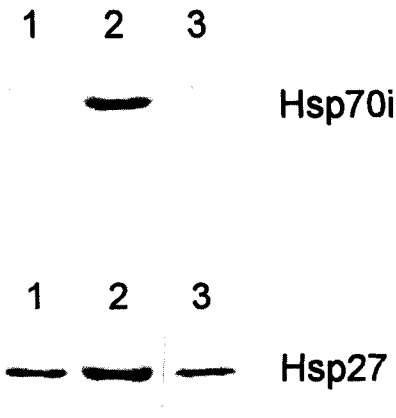

Fig. 5. Heat shock-induced accumulation of Hsp70i and Hsp27 in thermotolerant EC and inhibitory effect of quercetin. ECL blots demonstrate the antigen bands in sample aliquots from equal numbers of cells (3×10^5). (1) Non-tolerant cells; (2) cells rendered thermotolerant after heat shock (45°C, 10 min) and 14 h recovery at 37°C in growth medium; (3) cells preheated as in (2) and then incubated with 30 μM quercetin (Serva) for the first 6 h of post-heat shock recovery.

heat-shocked EC 30 μM quercetin, which blocks the heat shock gene transcription [29] and thereby abolishes cytoprotection mediated by inducible Hsps [30]. Fig. 5 shows that quercetin prevented the Hsp accumulation after heat shock. Furthermore, this reagent wholly abolished in preheated EC both the development of thermoresistance and the attenuation of the ATP drop-evoked changes in the Hsp27 status. All the beneficial effects of heat preconditioning on F-actin and morphology of ATP-depleted EC were also nullified by quercetin. This suggests that the post-heat shock EC adaptation to energy deprivation including stabilization of microfilaments and longer preservation of Hsp27 in the initial (phosphorylated and soluble) state is mediated by accumulation of heat-inducible Hsp(s).

4. Discussion

In the present work, we have shown that the PP inhibitors and the heat pretreatment exert similar effects on ATP-depleted EC, namely (i) arrest of the Hsp27 dephosphorylation/insolubilization/granulation, (ii) protection of F-actin from fragmentation and aggregation, and (iii) preservation of normal morphology and integrity of cell monolayer. We think all these effects are interrelated. No doubt, the increased stability of the cytoskeleton in the PP inhibitor-treated or thermotolerant EC determines their morphological tolerance to the ATP-depleting stress. With regard to Hsp27, the inhibition of its dephosphorylation upon ATP depletion is sufficient to abolish the insolubilization and intranuclear granulation (see Figs. 2B and 3E); however, it is yet unknown whether this event alone is sufficient for the cytoprotection observed. Retardation in dephosphophorylation of other cellular proteins (if it occurs) may also be significant for blocking the cytoskeletal and morphological perturbations in ATP-deprived EC. It was reported in 1992 that two inhibitors of PP1 and PP2A, calyculin A and okadaic acid, not preventing the ATP drop reduced the osmotic fragility and death of ischemia-stressed rat myocytes [31]. In our model, inhibition of PP1 by okadaic acid may be beneficial for endothelial F-actin, since increased activity of PP1 has been shown to bring about the dephosphorylation of myosin light chain and the disassembly of microfilaments in non-muscle cells [32]. PP2A, a major target for okadaic acid and cantharidin, appears to be responsible for the dephosphorylation of Hsp27 in vivo [25]. On the one hand, the discovered arrest of the Hsp27 dephosphorylation during ATP depletion may be a particular case of a wider phenomenon (i.e. the reduced dephosphorylation of many proteins) that somewhat ensures cytoprotection under energy deprivation. On the other hand, the artificial maintenance of the cytosolic (soluble) pool of phosphorylated Hsp27 within ATP-depleted EC may by itself contribute to the microfilament stability. In support of the latter, phosphorylated Hsp27 only was shown to be involved in protection of F-actin under heat shock [14,15], oxidative stress [16,17], and treatment with cytochalasin D [14,18], whereas non-phosphorylated Hsp27 promotes the reduction of F-actin in cells [12,13]. The precise mechanism of the protective action of sodium orthovanadate on ATP-depleted EC is unclear. Being an inhibitor of tyrosine PP this reagent may exert multiple effects on different cellular systems including the cytoskeleton. In our model, the suppression of the Hsp27 dephosphorylation by sodium orthovanadate appears to be due to the inhibition of a tyrosine PP involved in down-regulation of PP2A [33].

It is well known that non-lethal heat shock induces in mammalian cells the Hsp-mediated resistance to ATP depletion or ischemia, or anoxia (reviewed in [2]), though the machinery of this cross-tolerance remains to be clarified. Probably, tolerant cells enriched by Hsp70i can maintain the integrity of the cytoskeleton during ATP depletion [7–10] but no apparent ties between Hsp70 and microfilaments were revealed in ATP-deprived EC [2]. Nonetheless, the blocking action of quercetin indicates that the revealed effects of heat preconditioning on both Hsp27 and F-actin, and morphology of ATP-depleted EC are mediated by stress-inducible Hsp(s). Besides Hsp70i, prior heat shock increased the intracellular content of other Hsps including Hsp27 (Fig. 5). Martin et al. [34] have demonstrated that a rise in endogenous Hsp27 alone improves survival of rat cardiomyocytes undergoing simulated ischemia. However, it is poorly understood why excess Hsp27 within thermotolerant EC less undergoes the ATP drop-associated modifications (see Figs. 1–3). Speculating about the Hsp-mediated resistance of the cytoskeleton to lack of ATP we suggest that Hsp70i and/or other inducible Hsps can somehow block protein dephosphorylation during ATP depletion. In other words, inducible Hsps simulate the action of PP inhibitors that protects F-actin in case of energy deprivation. While mechanisms of the Hsp-mediated antiischemic defence are still a mystery, the suggested ability of excess Hsp(s) to neutralize the unbalanced PP activity within ATP-depleted cells seems to be quite an attractive idea.

In conclusion, we would like to note a medical aspect of our study. In vivo, depletion of cellular ATP can take place under acute ischemia and in case of surgical operations connected with bypass perfusion or transplantation of organs. The F-actin collapse within ATP-deprived EC leads to dysfunction of the endothelial barrier that aggravates ischemic lesions [23,24]. Evidently, EC tolerance to lack of ATP should improve outcome of an ischemic onset as well as recovery after 'surgical stress'. Our results allow us to point out two potential ways for protection of ATP-depleted EC: (i) usage of cell-permeant PP inhibitors and (ii) heat preconditioning to ele-

vate the intracellular Hsp content. The former seems to be a basis for creation of novel antiischemic drugs. In turn, the latter may find a use as the adapting pretreatment of isolated donor organs which are intended for implantation.

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