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Resveratrol Induces the Heat-Shock Response and Protects Human Cells from Severe Heat Stress

ÁKOS PUTICS, ESZTER MÁRIA VÉGH, PÉTER CSERMELY, and CSABA SŐTI

ABSTRACT

Molecular chaperones play key roles in protein quality control, signal transduction, proliferation, and cell death, and confer cytoprotection and assure survival after environmental stress. The heat-shock response is implicated in a variety of conditions including ischemic diseases, infection and immunity, neurodegeneration, and aging. Physiologic and pharmacologic chaperone inducers were shown to be an efficient therapeutic approach in different acute and chronic diseases. Here we characterize resveratrol, a polyphenol from red wine, as an inducer of the heat-shock response. Resveratrol activated the heat-shock promoter and the expression of the major chaperone Hsp70 in cell lines and in human peripheral lymphocytes, comparable to moderate heat stress. This effect was not due to its antioxidant property, because 5 m*M* *N*-acetylcysteine was unable to activate the heat-shock response. Moreover, resveratrol failed to upregulate Grp78, and tunicamycin was unable to induce Hsp70, suggesting that the resveratrol-induced heat-shock response was not mediated by canonic endoplasmic reticulum stress. Resveratrol synergized with mild to moderate heat shock and conferred cytoprotection against severe heat stress. Our results reveal resveratrol as a chaperone inducer that may contribute to its pleiotropic effects in ameliorating stress and promoting longevity. *Antioxid. Redox Signal.* 10, 65–75.

INTRODUCTION

MOLECULAR CHAPERONES are conserved, abundant, and essential proteins that guard the conformational homeostasis of proteins (15, 58). Chaperones in the everyday setting maintain signal transduction and other molecular networks, regulate proliferation and differentiation, and they are important modulators of the immune response and apoptotic pathways (9, 30, 44, 45). Besides, chaperone (or stress) protein levels are subject to a sudden and transient increase with stress, a so-called stress or heat-shock response, a basic cellular adaptation mechanism (27). Chaperone induction is mediated at the transcriptional level by an autoregulatory feedback loop: an increase in misfolded proteins results in the release of heat-shock transcription factor 1 (HSF-1)[1] from the repressing Hsp90/Hsp70/Hsp40 complex, and a consequent transcriptional activation of various heat-shock genes. Beyond chaperones, a variety of regulatory mechanisms are involved in the regulation of HSF-1 activity, including phosphorylation, trimerization, nuclear import, and termination of transcription (52).

The heat-shock response confers stress tolerance, cytoprotection, and assures short- and long-term survival during severe environmental conditions. Both a single heat shock and transgenic Hsp70 induce longevity (47). HSF-1 overexpression induces a twofold life-span extension, whereas HSF-1 knockout markedly shortens the life span (13, 18) and compromises immune responses in invertebrate and vertebrate model organisms, respectively (13, 57). Moreover, proteotoxicity and cellular degeneration increase, whereas chaperone inducibility and chaperone function decrease during aging (3, 28, 29). Modulation of the chaperone levels has been shown to provide protection in a variety of pathophysiologic states, such as ischemia/reperfusion, inflammation/sepsis, and conformational diseases (16, 41, 46, 49). Thus, preservation or enhancement of the heat-

Department of Medical Chemistry, Semmelweis University, Budapest, Hungary.

shock response is a subject of intense research. Chaperone induction has been proven in preclinical studies to be an efficient therapeutic approach in cardiovascular and age-related degenerative diseases involving cancer, diabetes, and neurodegeneration (11, 42, 43). Besides physiologic stressors (exercise, sauna, calorie restriction), both synthetic and plant-derived small molecules are among promising lead compounds (4, 19, 21, 23, 40, 50).

Resveratrol, a plant-derived polyphenolic compound, was identified in 1992 as an ingredient of grape skin and red wine, reducing the risk of coronary heart disease (40). Since then, it has become clear that resveratrol displays an impressive therapeutic potential against cancer, ischemic injuries, cardiovascular and inflammatory diseases, as well as neurodegeneration (5, 20, 33). A potent antioxidant and antiinflammatory property is an important determinant of its mechanism of action. Besides, recent studies have shown that it induces stress resistance and longevity in a variety of organisms, such as yeast, invertebrates, and vertebrates (17, 48), and it may reprogram the bodily processes similar to calorie restriction, probably by acting on the sirtuin (SIR, silent information regulator) family of deacetylases (56). The puzzling similarity between the therapeutic benefits of chaperone induction and resveratrol has led us to explore the effect of resveratrol on the heat-shock response of mammalian cells. Here we provide evidence that resveratrol activates the stress response, exerts a synergistic effect with mild to moderate heat treatment, and confers cytoprotection against a lethal heat shock. Our results reveal resveratrol as a chaperone inducer that may contribute to its pleiotropic effects in ameliorating stress and inducing longevity.

MATERIALS AND METHODS

Materials

Reagents for cell culture and Lipofectamine transfection reagent were from Gibco-Invitrogen (Carlsbad, CA); solutions and antibodies for flow cytometry were from BD Biosciences (San Diego, CA); and Ficoll-Paque PLUS was from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). The hsp70.1pr/luciferase plasmid was a kind gift of Richard Morimoto (Evanston, IL), the CMV/β-galactosidase was from BD Biosciences. The Bright-Glow luciferase and the β-galactosidase assay kits were from Promega (Madison, WI). Materials for protein determination, gel electrophoresis, and Western blotting were from Bio-Rad (Hercules, CA). *Trans*-resveratrol (RSV), *N*-acetyl-L-cysteine (NAC), hydrogen peroxide (H_2O_2), tunicamycin, the anti–β-actin antibody and propidium iodide were from Sigma (St. Louis, MO). The anti-Hsp70 fluorescein isothiocyanate (FITC) conjugated antibody (recognizing inducible Hsp70) and the anti-Grp78 mouse monoclonal antibodies were from StressGen (Victoria, BC, Canada). Peroxidase-conjugated secondary antibodies were obtained from DAKO Cytomation (Glostrup, Denmark), and the ECL reagent was from New England Nuclear, Perkin Elmer Life Sciences (Boston, MA). Complete tablets were from Roche (Basel, Switzerland). All other reagents were from either Sigma or Fluka (Buchs, Switzerland).

Preparation of lymphocytes

Human peripheral lymphocytes were isolated from a mixture of buffy coats (purchased from the Hungarian National Blood Transfusion Service, approved by the National Ethical Committee, and in accordance with the Helsinki regulations), which were usually prepared from the venous blood of five young healthy donors. To obtain leukocyte-rich plasma, the buffy coats were first layered over 6% dextran in a ratio of 9:1 (vol/vol) and sedimented for 1 h at room temperature. Lymphocytes were then isolated by Ficoll-Paque PLUS (d = 1.077 g/ml) gradient centrifugation, according to the manufacturer's instructions, and washed twice in phosphate-buffered saline (PBS). Aliquots were made in RPMI-1640 medium containing 40% fetal bovine serum and 5% DMSO and were frozen in liquid nitrogen until further use.

Cell culture and treatments

Peripheral lymphocytes were gently thawed and cultured in RPMI 1640 medium, supplemented with 10 m*M* Hepes, 10% fetal bovine serum, 2 m*M* L-glutamine, 100 μg/ml streptomycin, and 100 IU/ml penicillin at 37°C in a 5% CO_2 incubator at isobaric oxygen. The African green monkey kidney fibroblast-like cell line COS-7 was cultured in DMEM 4500, whereas the human cervical carcinoma cell line HeLa was cultured in minimal essential medium supplemented with 10% fetal bovine serum, 2 m*M* L-glutamine, 100 μg/ml streptomycin, and 100 IU/ml penicillin. For each experiment, cells were treated first with resveratrol (RSV) or with *N*-acetyl-L-cysteine (NAC) at 37°C in a 5% CO_2 incubator for 10 h. Subsequent treatments were performed with hydrogen peroxide (H_2O_2) for 2 h or with tunicamycin for 16 h at 37°C in a 5% CO_2 incubator. Heat shock was carried out at 41–45°C for 20–60 min either in an Eppendorf thermomixer (lymphocytes), or in a circulating water bath (cultured adherent cells).

Transfection and HSF-1 reporter gene assay

COS-7 cells were plated in six-well plates at a density of 20% and transfected the next day with 1 and 0.5 μg of hsp70.1pr/luciferase and CMV/β-galactosidase plasmids, respectively, by Lipofectamin, according to the manufacturer's protocol. Treatments were made 24 h after transfection in complete medium. Eighteen-hour posttreatment cells were lysed in β-galactosidase lysis buffer (Promega), and activities were determined by Bright-Glow luciferase and β-galactosidase commercial assay kits and were expressed as a ratio.

Determination of Hsp70 levels by flow cytometry

The 10^6 lymphocytes or COS-7 cells were treated either with resveratrol or NAC for 10 h and were or were not heat shocked, as specified in the figure legends. The induction of the heat-shock response was allowed to develop for 4 h at 37°C. Then cells were fixed and permeabilized in 250 μl Cytofix/Cytoperm solution at 4°C for 20 min. Cells were then washed twice with Perm/Wash solution and incubated with an anti-Hsp70 fluorescein isothiocyanate (FITC)-conjugated mouse monoclonal antibody and anti-CD3 Peridinin-chlorophyll-protein complex

(PerCP) conjugated antibody at 4°C for 30 min in Perm/Wash solution at a concentration recommended by the manufacturer. For compensation, cells were stained either with anti-CD3-PerCP–conjugated antibody or with anti-CD3 FITC–conjugated antibody. After washing once with Perm/Wash solution and once with PBS with 2% FBS, cells were fixed with 2% paraformaldehyde in PBS and analyzed with a FACSCalibur flow cytometer by using the CellQuest software (BD Biosciences). Approximately 10,000 live lymphocytes from each sample were electronically gated according to granularity and size in the forward *versus* side scatter. Differentiation between $CD3^+$ and $CD3^-$ subpopulations was achieved in the CD3-PerCP versus side scattergram. Hsp70-related fluorescence was obtained in a 530/30 (FITC) filter, and the relative Hsp70 protein level was expressed as the mean fluorescence intensity of FITC from logarithmic histograms.

Cell lysis and Western blotting

Cells were harvested, washed twice in PBS, then lysed in lysis buffer (20 m*M* Hepes, 100 m*M* NaCl, 1 m*M* EDTA, 1% NP40, pH 7.5, and 2× Complete protease inhibitor cocktail for 20 min at 4°C, vigorously vortexed, and centrifuged at 13,000 rpm for 10 min in a microcentrifuge. Protein content of the supernatants was determined by the Bio-Rad Bradford-assay with bovine serum albumin used as standard. Then 50 μg protein from detergent cell lysates was subjected to a 9% SDS-PAGE and electroblotted by using a semidry transfer apparatus on nitrocellulose membranes. Blots were blocked in 5% milk powder at room temperature, probed with the appropriate primary antibodies (Abs) overnight at 4°C, washed and incubated with peroxidase-conjugated secondary antibodies for an hour at room temperature, and developed by using an ECL reagent.

Cell-survival assay

The 10^6 cells per sample were incubated in the presence or absence of resveratrol for 10 h at 37°C, then treated with H_2O_2 or heat shocked, as indicated. Twenty-four hours later, cells were harvested, washed twice with PBS with 2% FCS, and stained with propidium iodide at a final concentration of 10 μg/ml in PBS with 2% fetal bovine serum at room temperature for 15 min. Flow-cytometric analysis was performed with a FACSCalibur flow cytometer and the CellQuest software by using 488 nm excitation and 630/22 emission filters, respectively. Cell debris was eliminated by gating according to side-scatter and forward-scatter detection, and ~10,000 cells were analyzed. The percentage of PI-negative (surviving) cells was calculated by single-parameter analysis of the PI-related fluorescence by using logarithmic histograms.

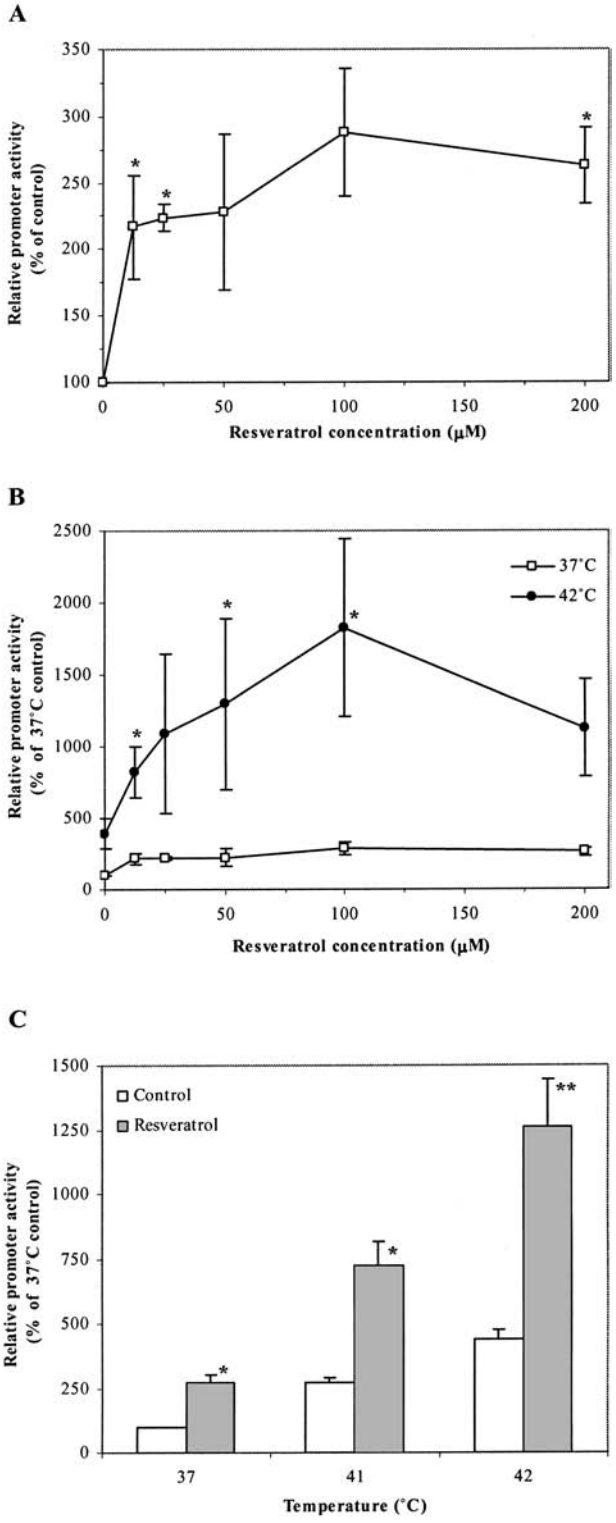

FIG. 1. Resveratrol induces heat-shock promoter activation and acts as a chaperone co-inducer. (**A**) Activation of the hsp70.1 promoter reporter by resveratrol at 37°C. (**B**) Resveratrol potentiates the heat-shock response in a concentration-dependent manner (the 37°C curve is displayed for comparison). (**C**) Resveratrol lowers the threshold of the heat-shock response. COS-7 cells were co-transfected with hsp70.1pr/luciferase and CMV/β-galactosidase and were incubated with the indicated concentrations of (**A, B**) or 50 μM (**C**) resveratrol for 3 h, and then were heat stressed for 20 min at the indicated temperatures (**B, C**) as described in Materials and Methods. After 18 h, enzyme activities were determined, and their ratio was expressed. Data represent mean ± SD of three experiments. * or **, significant differences at $p < 0.05$ or $p < 0.01$, respectively.

Statistical analysis

Data were statistically analyzed by using Statistical Package for the Social Sciences software version 15.0 for Windows (SPSS Inc., Chicago, IL). Variables are expressed as mean ± standard deviation (SD). Means were compared by using the Student's *t* test. A *p* value (two-tailed) < 0.05 was considered statistically significant.

RESULTS

Resveratrol activates heat-shock promoter–driven transcription

To investigate the effect of resveratrol on the stress response, we first asked whether it induced the activation of the heat-shock promoter containing consensus binding motifs of HSF-1. We transiently transfected COS-7 cells with a luciferase construct fused to the promoter of the major stress protein, the inducible isoform of Hsp70 (53) and observed a dose-dependent induction of the reporter activity at both 37°C and at 42°C (Fig. 1A and B). The EC_{50} values calculated from double reciprocal plots were 8.6 and 15.2 μM, respectively. A cytotoxic effect was already observed at 200 μM and became predominant at 400 μM, where the stress-response induction was compromised, as well (data not shown). Resveratrol potently induced heat-shock promoter–driven transcription in HeLa cells, as well (data not shown).

Because resveratrol potentiated the heat-shock response at 42°C (Fig. 1B), we tested whether they cooperate in a synergistic manner. To this end, cells were pretreated with resveratrol and subjected to heat stress at different temperatures. Figure 1C shows that resveratrol exerted an effect comparable to

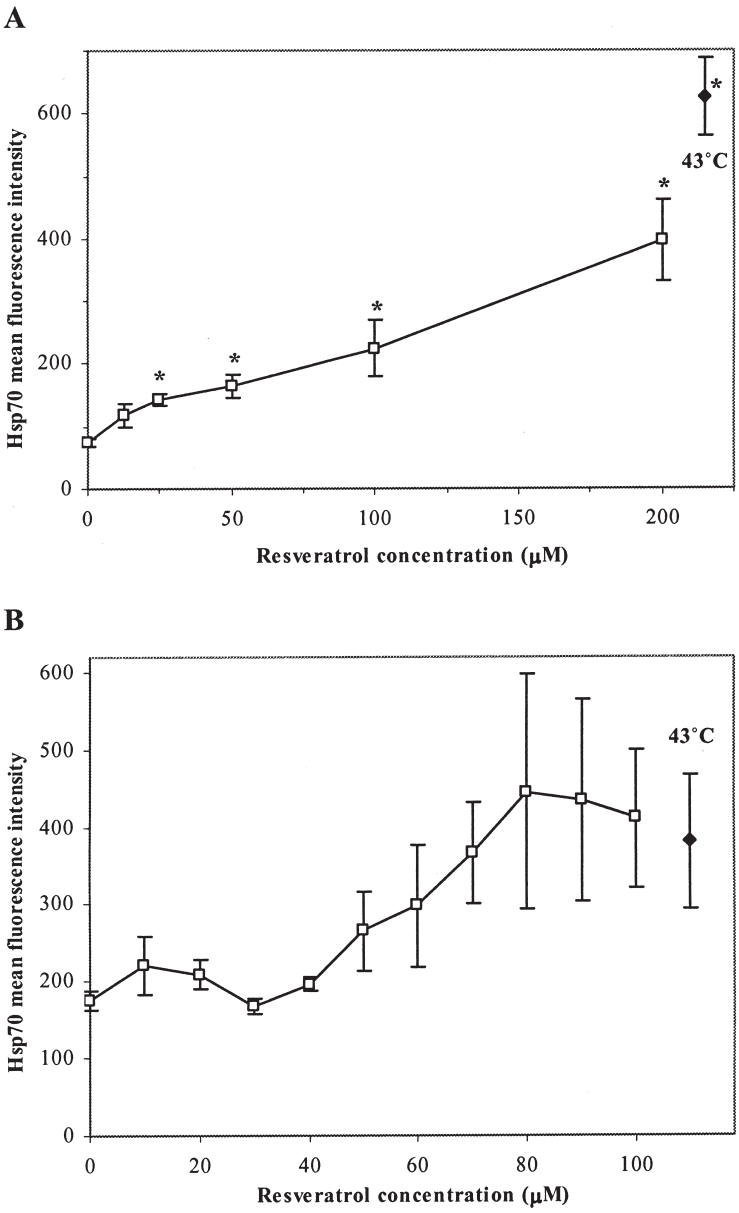

FIG. 2. Resveratrol induces Hsp70 protein expression in different cell types. COS-7 cells (**A**) or human peripheral lymphocytes (**B**) were incubated with the indicated concentrations of resveratrol for 15 h; then cells were harvested, stained with an anti-Hsp70 antibody FITC-conjugate, subjected to flow cytometry, and the mean fluorescence intensities were determined, as described in Materials and Methods. *Solid diamonds*, A 20-min (**A**) or a 60-min (**B**) heat stress at 43°C 4 h before analysis. Data represent mean ± SD of three (**A**) or two (**B**) experiments, respectively. *Significant difference at $p < 0.05$.

a 41°C heat shock, and a combination treatment at 41°C resulted in a higher heat-shock response than that at 42°C. Similarly, the combination of resveratrol with a 42°C treatment was highly synergistic, suggesting that resveratrol increases the robustness of adaptation to a harmful stimulus. HeLa cells similarly displayed a very pronounced synergy between resveratrol and heat shock (data not shown).

Resveratrol induces Hsp70 protein expression

To see whether the activating property of resveratrol would be translated into the expression of intrinsic chaperone proteins, COS-7 cells were treated with resveratrol, and the amount of the inducible Hsp70 protein was analyzed with flow cytometry (Fig. 2A). Resveratrol induced a fivefold accumulation of Hsp70 protein, close but not similar to the protein level after a 43°C heat stress. Maximal induction could not be properly determined because above 200 μM, resveratrol became cytotoxic (data not shown). After establishing resveratrol as a chaperone inducer in cell lines, we were seeking a more physiologic cellular model to characterize. Because the stress response is of central importance in the immune system, lymphocytes are governors of immune activity, show a weak stress response, and heat stress in the form of fever potentiates their function (30, 31, 38), we investigated human peripheral lymphocytes as a possible target of the action of resveratrol. Indeed, resveratrol treatment recapitulated the earlier findings on cell lines, although with different kinetics (Fig. 2B). Hsp70 induction seemed to start at the higher dose of 40 μM and reached a plateau at 80 μM, resembling to a sigmoid cooperative response curve. Even though none of the values, including the heat stress, reached statistical significance, the maximal Hsp70 induction of resveratrol tended to be higher than that of the 43°C treatment, suggesting a physiologically relevant stress response–inducing property.

Resveratrol's mechanism of action is mediated neither by its antioxidant property nor by a canonic ER stress response

A potent direct antioxidant property is a reminiscent feature of polyphenols and contributes to the beneficial effects of resveratrol. However, an increasing body of evidence argues for an indirect effect and implicates the upregulation of antioxidant enzymes and inhibition of redox-active signaling by resveratrol (35). It was also reported that the thiol antioxidant 1,2-dithiole-3-thione stimulated Hsp70 expression in dopaminergic neuronal cells (2). To mimic the presumed antioxidant property of resveratrol, we examined the possibility that a well-known thiol antioxidant, *N*-acetylcysteine (NAC) (14) would interfere with the stress response. But 5 m*M* NAC was unable to activate or coactivate the heat-shock promoter with heat stress in COS-7 and HeLa cells, respectively (Fig. 3A and data not shown). In contrast, it inhibited the heat-induced reporter activity, suggesting the role of reactive oxygen species in promoter activation. However, when H_2O_2 was tested at various concentrations, it did not induce the stress response at the HSF-1, the mRNA, or the protein level (E.M.V, Á.P., C.S., unpublished observations). Moreover, the inhibitory effect of NAC could not be observed in HeLa cells, arguing against a general mechanism (data not shown). Besides the results shown here, NAC had no influence on Hsp70 protein expression either in COS-7 cells or in peripheral lymphocytes (Fig. 3B and data not shown), which further argues against a general antioxidant property of resveratrol in its heat-shock–related effects.

It was recently reported that resveratrol induces longevity in *Caenorhabditis elegans via* the induction of a subset of ER-

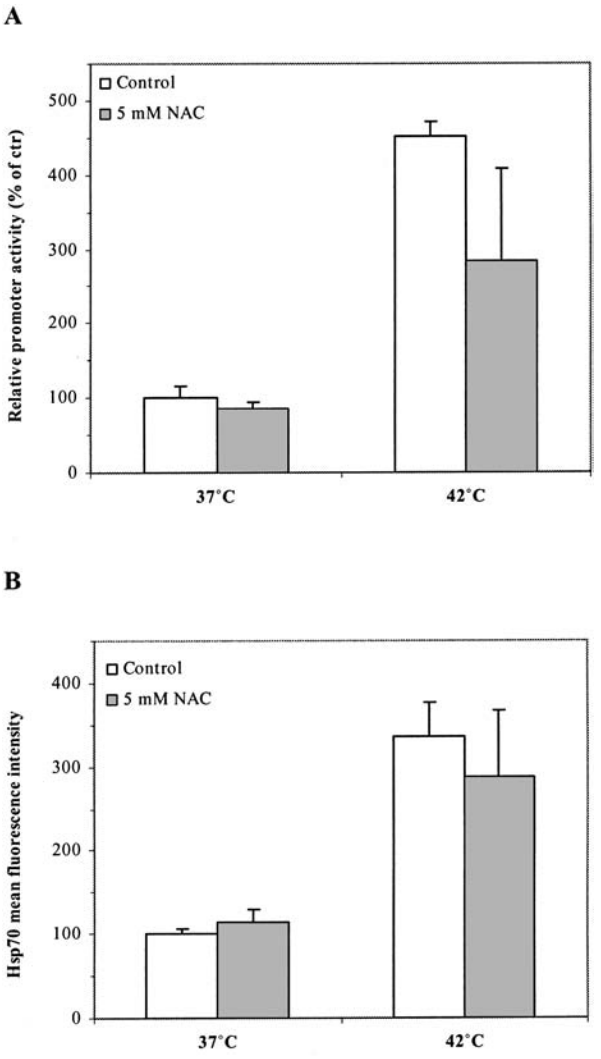

FIG. 3. The antioxidant *N*-acetylcysteine does not induce the heat shock response. (**A**) *N*-acetylcysteine (NAC) does not activate the hsp70.1-promoter reporter. COS-7 cells were co-transfected with hsp70.1pr/luciferase and CMV/β-galactosidase and were incubated with or without 5 m*M* NAC for 3 h, and then were heat stressed at 42°C or were kept at 37°C for 20 min, as described in Materials and Methods. After 18 h, enzyme activities were determined, and their ratio was expressed. (**B**) NAC does not induce Hsp70 protein expression. COS-7 cells were incubated with or without 5 m*M* NAC for 10 h, were heat stressed at 42°C, or were kept at 37°C for 20 min, as described in Materials and Methods. Four hours later, cells were harvested, stained with an anti-Hsp70 antibody FITC-conjugate, subjected to flow cytometry, and the mean fluorescence intensities were determined. Data represent mean ± SD of two experiments. *Significant difference at $p < 0.05$.

resident protein genes (51). Therefore, we analyzed possible crosstalk between the unfolded protein response/ER-stress pathway and the cytosolic heat-shock response. We found that tunicamycin, an inhibitor of core glycosylation, did not activate the heat-shock reporter. Furthermore, tunicamycin potently inhibited the heat-induced activation of the heat-shock promoter in both COS-7 and HeLa cells (Fig. 4A and data not shown). Notably, Hsp70 protein levels did not reflect the inhibition of the heat-shock promoter on tunicamycin treatment (Fig. 4B), raising the possibility that in these circumstances, transcriptional and posttranscriptional regulation of Hsp70 became uncoupled, which resulted in the preservation of adaptation to heat stress.

To acquire an insight into the effect of resveratrol on ER stress, Grp78, the major chaperone of the ER and a marker of the unfolded protein response, was analyzed with Western blotting. Figure 4C shows that neither resveratrol nor NAC induced Grp78 protein. Furthermore, neither agent interfered with Grp78 induction on tunicamycin treatment. Our results confirm earlier findings on *C. elegans*, in which resveratrol did not induce a canonic unfolded protein response, including the Grp78 orthologue (51). Neither they nor we found a general derangement in protein homeostasis in the models studied on resveratrol treatment (51, and data not shown). These observations suggest neither an overwhelming stress nor an imbalance in protein homeostasis, but a specific signaling event may mediate a selective induction of the cytosolic stress response by resveratrol.

Resveratrol protects cells from severe heat stress

Resveratrol has a well-documented chemopreventive and cytoprotective property because of its prominent action on free radicals and on the redox homeostasis (5, 35). Resveratrol pretreatment potently inhibited cell death under circumstances of oxidative stress in human peripheral lymphocytes (Fig. 5A). However, its ability to induce apoptosis may mask its beneficial effects under other stresses (12). We observed an augmented cell death in resveratrol-treated lymphocytes compared with control cells (data not shown).

Because molecular chaperones and the heat-shock response antagonize proteotoxic noxae, we tested whether resveratrol conferred cytoprotection against heat stress. Cells preconditioned with resveratrol displayed an increasing survival at increasing temperatures, even at the lethal 45°C heat shock (Fig.

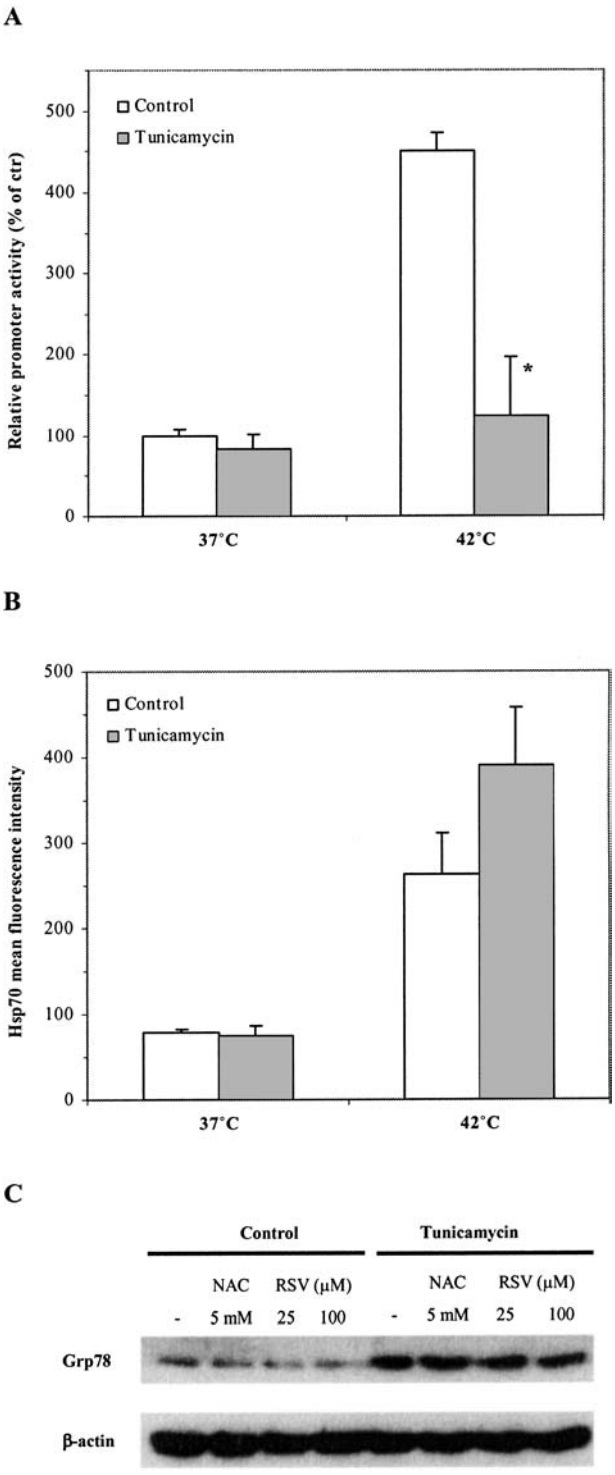

FIG. 4. Resveratrol does not use a canonic ER-stress pathway to induce the heat-shock response. (**A**) Tunicamycin does not activate the hsp70.1–promoter reporter and inhibits its heat-induced activation. COS-7 cells were co-transfected with hsp70.1pr/luciferase and CMV/β-galactosidase, and were incubated with or without 2 μg/ml tunicamycin for 3 h, and then were heat stressed at 42°C or were kept at 37°C for 20 min, as described in Materials and Methods. Eighteen hours later, enzyme activities were determined, and their ratio was expressed. (**B**) Tunicamycin does not induce Hsp70 protein expression. COS-7 cells were incubated with or without 2 μg/ml tunicamycin for 10 h, were heat stressed at 42°C, or were kept at 37°C for 20 min. Four hours later, cells were harvested, stained with an anti-Hsp70 antibody FITC-conjugate, subjected to flow cytometry, and the mean fluorescence intensities were determined. (**C**) Resveratrol does not induce Grp78 protein expression. COS-7 cells were incubated with or without NAC and resveratrol (RSV) at the indicated concentrations for 3 h, and then samples were supplemented with 1 μg/ml tunicamycin, and incubation was continued for 15 h. Cells were harvested, lysed, and subjected to immunoblotting for Grp78 and β-actin. Data represent mean $\pm$ SD, and the blot is a representative of two experiments. *Significant difference at $p < 0.05$.

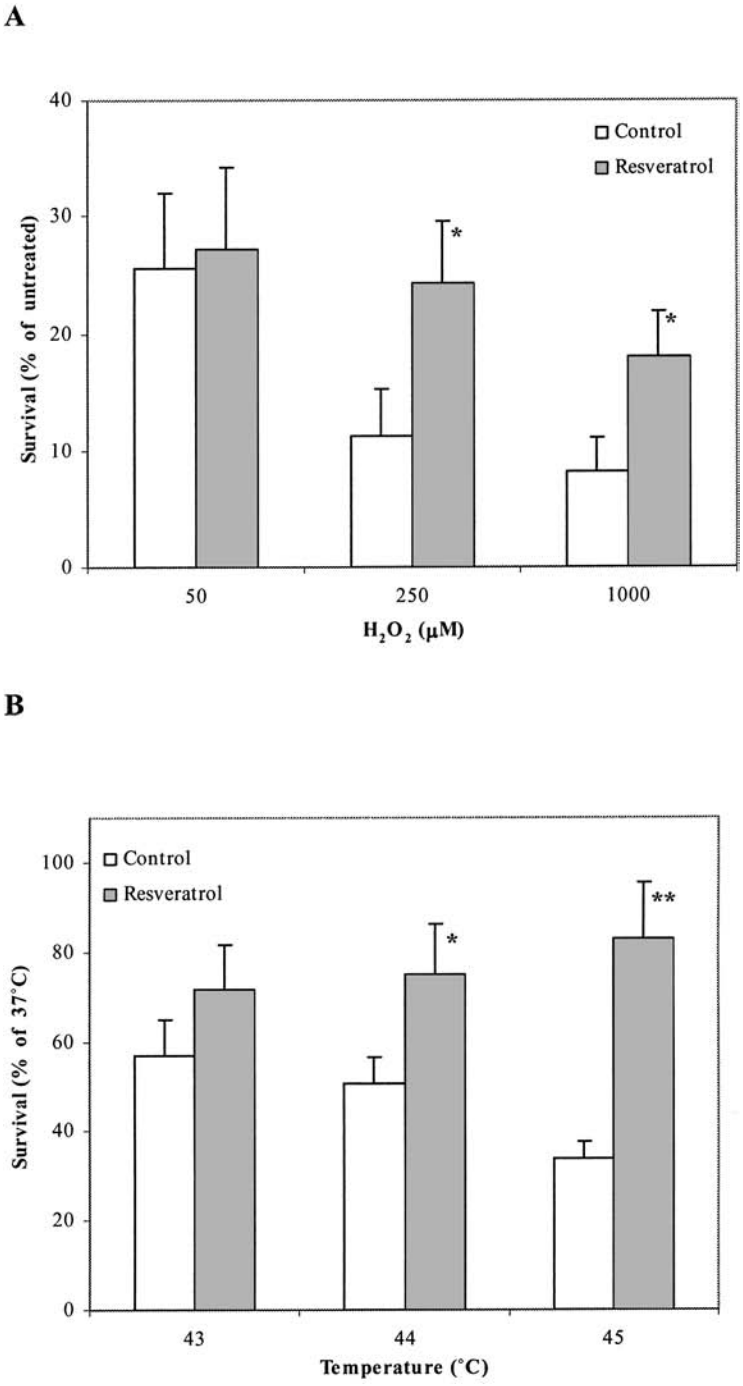

FIG. 5. Resveratrol confers cytoprotection against oxidative and heat stress. (**A**) Resveratrol protects the cells from hydrogen peroxide. (**B**) Resveratrol protects the cells from heat stress. Lymphocytes were incubated in the presence or absence of 100 μM resveratrol for 10 h at 37°C then treated with H_2O_2 for 2 h or heat shocked at the indicated temperatures for 30 min. After 24 h, cells were incubated with propidium-iodide and analyzed in a flow cytometer. The fraction of the propidium-iodide–negative (surviving) cells was expressed relative to the absolute and resveratrol-treated controls. Data represent mean ± SD of three experiments. *, **Significant differences at $p < 0.05$ or $p < 0.01$, respectively.

5B). These results suggest that resveratrol may ameliorate the consequences of proteotoxicity, probably *via* induction of the heat-shock response.

DISCUSSION

Resveratrol is a member of the polyphenol family of phytochemicals, plant-derived small molecules with potent and wide range of biological effects. Polyphenols, including resveratrol, possess antimicrobial, antitumor, antiinflammatory, antiischemic, and neuroprotective properties (12, 35, 36). Resveratrol is the first compound that has been recognized to promote longevity in several organisms (17, 48, 56), including mice kept on a high-calorie diet (6), predicting a tremendous benefit for human health. It has also been suggested that resveratrol acts *via* the sirtuin family of the NAD^+-dependent deacetylase family that connects metabolic stress and aging (17).

Our study identified resveratrol as an inducer of the heat-shock response. Resveratrol treatment of various mammalian, including human, peripheral cells activated HSF-1–dependent

transcription and accumulation of Hsp70 protein at a level comparable to the effect of a moderate heat stress. The EC_{50} value was in the low micromolar range (~10 μM), comparable to that of the activating effect on sirtuins (56). However, it is still much higher than the peak serum concentration after two glasses of red wine [2.4 n*M* unmodified and 180 n*M* derivatized resveratrol, respectively (5)]. These data recall a major obstacle of resveratrol research (*i.e.*, a gap between bioavailability and the effective concentration). In support of a physiologically relevant stress-inducing effect is the fact that use of a 100-mg/kg pharmacologic dose resulted in 9 μM unmodified and 680 μM derivatized resveratrol concentration, respectively (5). Furthermore, various organs can accumulate resveratrol 30-fold over the serum concentration; other polyphenols synergize with and inhibit the conjugation of resveratrol, and altogether they may put the red-wine consumption in the effective range.

The observed synergy of resveratrol with heat shock suggests that small or transient increases in resveratrol concentration may cooperate with other small stresses and promote the mounting of a more robust stress response. This is reminiscent of a chaperone co-inducer property (42, 50) and is highly beneficial, because reprogramming the cellular thermometer increases the stress tolerance and the fitness of the cell or organism.

By what mechanism does resveratrol induce the stress response? Studies with NAC strongly suggest that an antioxidant activity did not mediate this action. Moreover, our observations on protein aggregation and on the cytoprotective effect in case of simultaneous resveratrol and heat treatment argue against the possibility that resveratrol compromised global protein homeostasis (data not shown). In some circumstances, ER stress may induce cytosolic chaperones (32). In our model, the canonic unfolded protein response was unlikely to be involved in the resveratrol-induced stress response, because neither did tunicamycin activate the heat-shock response, nor did resveratrol induce the ER Hsp70 homologue UPR marker, Grp78. In other studies, resveratrol treatment seemed to upregulate the apoptosis-inducing transcription factor CHOP in colon carcinoma cells (54) and induced life-span extension through induction of a subset of nonclassic UPR genes (51), suggesting that resveratrol may interfere with and provide protection against stressful events in the ER. Thus, crosstalk between a noncanonic UPR and the cytosolic heat-shock response cannot be excluded.

Chaperone inducers may work along the heat-shock pathway: through direct binding and activation of HSF-1, inhibition of chaperones suppressing HSF-1, inhibition of the proteasome, or by other hitherto unknown mechanisms (4, 19, 21, 23, 42, 43, 50, 53). Alternatively, an interference with the phosphorylation of HSF-1 may also modulate the stress response, because protein kinase C, c-jun N-terminal kinase, and glycogen synthase kinase 3 all repress HSF-1 activation (52), and, in turn, a resveratrol-mediated inhibition of these kinases has been reported (34, 54). Interestingly, another phenolic acid derivate from cinnamon has recently been shown to activate the PI3-kinase Akt pathway (24) that exerts an inhibition on glycogen synthase kinase 3, suggesting a possible pathway for polyphenol-dependent HSF-1 activation.

Resveratrol belongs to the group of agents/stimuli that exhibit a reciprocal effect on HSF-1 activation (stress resistance) and NF-κB activation (inflammation). These processes have an opposite effect on longevity (5, 35, 53) and may be coordinately regulated branches of a survival program. This program is regulated by the level of environmental stress and operates with multiple sensors that transmit signals from the internal and external environment and use robust and redundant pathways that switch from costly reproduction to self-maintenance. One master regulator is the sirtuin family, which responds to food availability and may mediate the effects of calorie restriction (8, 22). It is tempting to speculate whether SIR2 is an upstream regulator of HSF-1. Evidence has suggested that SIR-2.1 may activate HSF-1 through the forkhead transcription factor Daf-16 in *C. elegans* (8). However, a direct inhibitory effect *via* chromatin structure was also described in yeast (39). In this context, resveratrol either may induce an SIR2-dependent activation or may inhibit a repression on the heat-shock response, or both (17, 51, 56). Whether resveratrol exclusively uses any of the pathways listed or promiscuously affects many of them simultaneously remains to be seen. Another exciting question is whether HSF-1 is needed for resveratrol to induce longevity, as was the case in daf-2 mutants (18).

Resveratrol exerted a cytotoxic effect both on lymphocytes and on cultured cell lines over 50 μM, accompanied by the downregulation of the stress response above 200 μM, in agreement of its proapoptotic activity (12, 54). Many chaperones exert a potent antiapoptotic effect, a possible escape and survival mechanism for tumor and normal cells, respectively (42, 43, 45). In this regard, the resveratrol-induced heat-shock response may be an aspecific compensatory mechanism in cytotoxicity. One study found a correlation between resveratrol treatment, apoptosis, and Hsp70 level in prostate cancer cells (7) that supports this hypothesis. In addition, the mechanistic insights presented in this study argue for a more specific mechanism of action.

Table 1. Effect of Resveratrol on the Stress Response and Thermotolerance in Various Mammalian Cells

	Lymphocyte	*COS-7*	*HeLa*
Heat-shock response			
Heat-shock promoter activation	N.D.	Yes	Yes
Synergy with heat shock	N.D.	Yes	Yes
Hsp70 protein induction	Yes	Yes	Yes
Thermotolerance	Yes	Yes	N.D.

N.D., not determined.

An important finding of the present work is that resveratrol protects cells from severe heat stress. It corroborates earlier results in which resveratrol rescued neuronal cells from mutant huntingtin proteotoxicity (33). In that study, polyglutamine toxicity was prevented by either SIR-2.1 overexpression or resveratrol treatment in *C. elegans*, suggesting that resveratrol may act *via* activation of SIR-2.1. Because HSF-1 is critical to combat aggregation (18, 28), we propose that resveratrol exerts its beneficial activity *via* the induction of the heat-shock response, a central protein quality-control mechanism. The conservation of this pathway in human cells is reasonable. Although HSF-1 maybe only one player in the signaling network that is regulated by the metabolic state, downregulation of the stress response may result in degenerative diseases (ischemic diseases, neurodegeneration, inflammation) as a consequence of overnutrition, all being major threats of Western civilization. Direct evidence indicates that hyperlipidemia blunts the stress response in the rat (10). Resveratrol, in contrast, lowers the temperature threshold of the heat-shock response, so cells become preconditioned to cope with a more severe, lethal stress, exactly what we have seen in the cytoprotection experiments. These effects were observed in various mammalian cell lines and peripheral cells, reflecting a conserved phenomenon (see Table 1).

Our data showed a biphasic effect of resveratrol on both heat shock–promoter activation and Hsp70 protein expression. This, as well as the cytotoxicity observed at higher doses, is a characteristic hormetic property (37). Hormesis is a dose–response phenomenon characterized by low-dose stimulation, high-dose inhibition. Similarly, a number of other phytochemicals, called hormetins, have been shown to evoke hormetic responses. The stress-response inducers curcumin and celastrol have both been demonstrated to induce the heat-shock response in a hormetic manner (1, 53).

The hormetic property of resveratrol also supports the "xenohormesis hypothesis," which postulates that small but harmful changes in the environment (famine, dryness, heat, cold, infection) induce the production of phytoalexins in plants (25). Most of these compounds are potent toxins, especially for insects (26), but their amount does not reach a critical threshold in bigger animals and promotes a hormetic response. Alternatively, phytoalexins seem to be danger signals and represent an altruistic courtesy of stressed plants toward ingesting animals, alerting them to prepare for and to survive during unfavorable environmental conditions. This principle was intuitively used for centuries in the form of herbal remedies by the ancient wisdom of folk therapy and of traditional oriental medicine. Indeed, xenohormetic compounds including resveratrol feature overlapping properties: they induce self-maintenance and protective mechanisms, such as improving energy metabolism (increasing insulin sensitivity), upregulation of phase II detoxification, antioxidant enzymes, and suppression of inflammatory pathways (26, 35), a key to a healthy life.

In conclusion, we propose that among other desired physiologic effects, resveratrol activates the stress response, a molecular pathway to combat stress, prevent disease, and induce longevity. Deeper understanding of the mechanism of action as well as the intricate interplay of resveratrol with various targets may lead to a better understanding of aging, stress, and longevity.

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ABBREVIATIONS

ER, endoplasmic reticulum; FITC, fluorescein isothiocyanate; Grp78, 78-kDa glucose-regulated protein; HSF-1, heat-shock transcription factor 1; Hsp, heat-shock or stress protein, the number thereafter denotes molecular weight in kilodaltons; NAC, *N*-acetyl-L-cysteine; PBMC, peripheral blood mononuclear cells, RSV, resveratrol (3,4′,5-Trihydroxy-transstilbene); SIR, silent information regulator; UPR, unfolded protein response.

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Address reprint requests to:
Csaba Sőti
Department of Medical Chemistry
Semmelweis University
P.O. Box 260
Budapest, H-1444, Hungary

E-mail: csaba@puskin.sote.hu

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