Chapter 19

TURNING UP THE HEAT IN THE LUNGS

A key mechanism to preserve their function

Claudio Sartori and Urs Scherrer

Abstract: Life threatening events cause important alterations in the structure of proteins creating the urgent need of repair to preserve function and ensure survival of the cell. In eukariotic cells, an intrinsic mechanism allows them to defend against external stress. Heat shock proteins are a group of highly preserved molecular chaperones, playing a crucial role in maintaining proper protein assembly, transport and function. Stress-induced upregulation of heat shock proteins provides a unique defense system to ensure survival and function of the cell in many organ systems during conditions such as high temperature, ischemia, hypoxia, inflammation, and exposure to endotoxin or reactive oxygen species. Induction of this cellular defense mechanism prior to imposing one of these noxious insults, allows the cell/organ to withstand a subsequent insult that would otherwise be lethal, a phenomenon referred to as "thermo-tolerance" or "preconditioning". In the lung, stress-induced heat shock protein synthesis, in addition to its cyto-protective and anti-inflammatory effect, helps to preserve vectorial ion transport and alveolar fluid clearance. In this review, we describe the function of heat shock proteins in the lung, with particular emphasis on their role in the pathophysiology of experimental pulmonary edema, and their potential beneficial effects in the prevention and/or treatment of this life-threatening disease in humans.

Key Words : heat shock proteins, lung, acute respiratory distress syndrome, alveolar fluid clearance, epithelial sodium channel

Hypoxia: Through the Lifecycle, edited by R.C. Roach *et al.*
Kluwer Academic/Plenum Publishers, New York, 2003.

STRESS-INDUCED PROTEIN DENATURATION INCREASES THE EXPRESSION OF HSP

In 1962 Ritossa observed that exposing *Drosophila* to elevations of temperature produced "puffing" patterns of polytene chromosomes indicating increased gene activity (18). Approximately 10 years later, Tissières and colleagues demonstrated that these "puffing" patterns represented upregulation of genes encoding for heat shock proteins (HSP) (26). This heat shock response, now commonly referred to as the stress response, is ubiquitous in nature and consists of the transcription and translation of a set of HSPs, which possess a tremendous homology across virtually all living cells.

HSPs are proteins ranging from 8–110 kDa that are assigned to families on the basis of sequence homology and typical molecular weight (33, 34). In eukaryotes, there exist many families that comprise multiple members, differing in degree and kinetics of inducibility, intracellular distribution, tissue specificity and function (3, 4).

Table 1. Heat shock protein families, localization and function

NAME	kDA	LOCALISATION	FUNCTION
Ubiquitin	8	Cytosol/nucleus	Degradation
HSP 27	27	Cytosol/nucleus	Molecular chaperone; cytoprotection
Heme Oxygenase	32	ER and cytoplasm	Resistance to oxidant stress
HSP 47	47	ER	Collagen chaperone
HSP 60	60	Mitochondria	Molecular chaperone
HSP 70	72	Cytosol/nucleus	Cytoprotection
HSP 90	90	Cytosol/nucleus	Regulation steroid receptor activity
HSP 110	110	Nucleolus/cytosol	Nucleoli protection from stress

MECHANISMS CAUSING INDUCTION OF HSP EXPRESSION

In addition to elevated temperatures, induction of HSP expression has also been observed under various other conditions such as ischemia, oxygen deprivation, inflammation, or exposure to endotoxin, reactive oxygen species, ethanol, heavy metals or other chemical denaturants. All these different forms of stress may induce protein conformational changes either directly or indirectly.

Accumulation of denatured or abnormally folded proteins itself is assumed to represent the key proximal signal for initiation of the stress response in a given cell or tissue (27). The exact underlying mechanisms by which denatured proteins initiate the stress response

are incompletely understood, but are thought to relate to the ability of denatured proteins in the cytoplasm to stimulate a cascade of interactions between heat shock protein and a series of co-chaperones such as heat shock factors (HSF) and heat shock elements (HSE) which finally results in activation of the HSP promoter and a dramatic and rapid increase in specific stress protein expression (4).

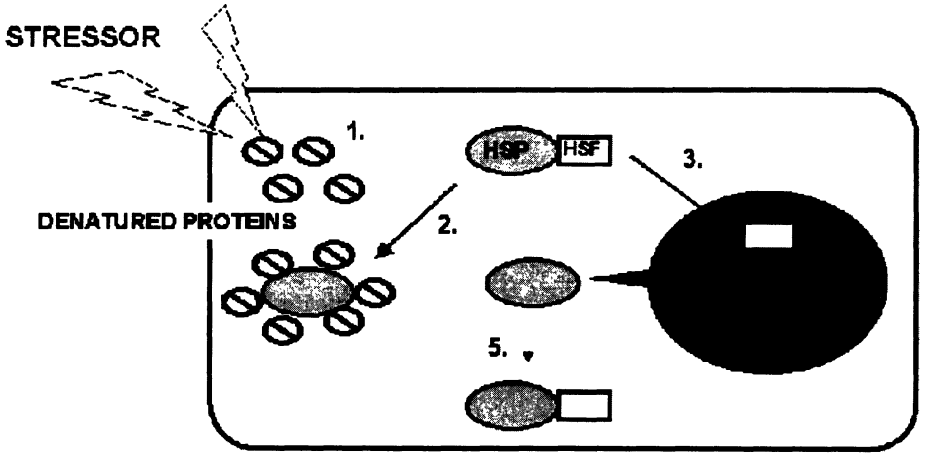

Figure 1. During stress, denatured proteins (1) are bound by existing intracellular pools of HSP70 (2), causing a relative depletion of unbound HSP70. The decreased level of intracellular HSP70 shifts the equilibrium between HSF and HSP70, thus liberating HSF to trimerize, translocate to the nucleus (3), and activate HSP70 transcription (4) via high-affinity binding with the HSE (heat shock elements). When the level of newly synthetized HSP70 reaches some critical level, the equilibrium between HSF and HSP70 is restored (5), and HSF activation is terminated. HSF can then translocate to the nucleus and interact with heat shock elements in the promoters of *HSP70* and other target genes.

Increased HSP mRNA transcripts are present already a few minutes after a stress occurs, whereas protein accumulation reaches its maximum roughly 12 hours after stress induction. Thereafter, HSP content in tissues slowly decreases, but may remain elevated up to 192 hours after the initial stimulus.

CYTOPROTECTIVE EFFECTS OF INCREASED HSP EXPRESSION

Although the precise function of the stress proteins is not known, it is clear from a number of studies that they have cytoprotective effects. Heating cells to a few degrees Celsius above their resting temperature for a short period of time confers protection a few hours later to a second heat stimulus that would otherwise be lethal: a phenomenon described as thermo-tolerance. Furthermore, heating also confers tolerance to other, non-thermal, noxious stimuli, and conversely, induction of the stress response by non-thermal means

can induce thermo-tolerance. The term cross-tolerance has been coined to describe this phenomenon (27, 40).

The mechanisms by which the stress response and stress proteins confer cytoprotection are still poorly understood. As molecular chaperones, stress proteins are known to transiently stabilize and refold damaged intracellular proteins and prevent intracellular protein aggregation during stress. Alternatively, several other protective functions have been attributed to HSPs.

An important feature of the stress response is that increased HSP expression is associated with a concomitant transient shut-down of non-stress protein gene expression. Based on this observation, it has been postulated that stress response-mediated inhibition of gene expression, particularly pro-inflammatory gene expression, may be one of the mechanisms by which the stress response protects against acute injury.

Protective effects of HSPs have also been attributed to their ability to 1) decrease the intracellular level of radical oxygen species (ROS, and, in turn, modulate glutathione metabolism to maintain it in reduced state 2) suppress apoptotic signaling pathways (inhibition of JNK-mediated apoptosis, inhibition of caspase activity); and (3) interact with nitric oxide-induced cytoprotection (4).

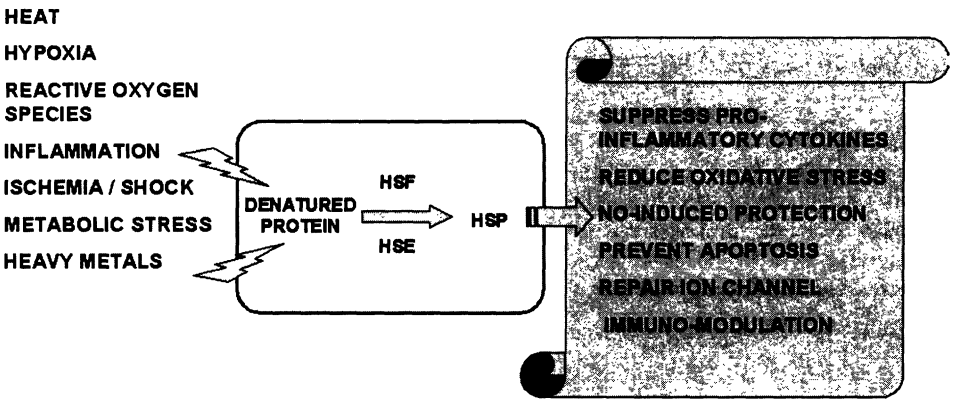

Figure 2. Proteotoxic stressors and cytoprotective effects of heat shock proteins

In summary, the stress response is a highly conserved evolutionary adaptation designed to quickly remove damaged proteins and restore the normal protein folding environment of cells following a proteotoxic insult. Even if not exclusively, this protection is largely attributable to induction of specific heat shock protein expression. Based on this concept, novel therapeutic strategies using pharmacologic interventions and/or gene transfection techniques are being investigated for their potential to enhance HSP expression by the cells. In cardiovascular disease such strategies have been intensively investigated to improve the tolerance of myocardial cells against ischemic insults and, thereby, improve the outcome and survival of patients suffering from ischemic events.

HSP IN THE LUNGS

That the stress response may also play a critical role in lung biology is easily predictable given its highly conserved nature. Surprisingly, however, its role has begun to be elucidated only very recently. Among the many classes of stress proteins, heme-oxygenase-1 (HO-1) and heat shock protein 70 are the best characterized with respect to lung biology (40). Hypoxia is a potent, but transient inducer of HO-1 in vascular smooth muscle cells *in vitro* and in the lung *in vivo* (6).

Stress protein expression has been well described in whole lungs and in specific lung cells from various species. Cultured pulmonary artery endothelial cells, airway epithelial cells, pulmonary artery smooth muscle cells and alveolar macrophages express abundant HSP70 after thermal stress (40). In patients suffering from cancer, asthma, or acute lung injury, augmented HSP expression has been reported in the lung *in vivo*.

STRESS PROTEINS HAVE AN IMPORTANT CYTOPROTECTIVE ROLE DURING LUNG INFLAMMATION AND INJURY

Five years after Ritossa's description of the heat-induced puffing patterns of polytene chromosomes in the Drosophila, Ashbaugh and colleagues described a new clinical syndrome that they called the acute respiratory distress syndrome (ARDS) (1).

ARDS is a form of non-cardiogenic pulmonary edema, associated with pulmonary infiltrates, stiff lungs, and severe hypoxemia which affects 50-75 per 100,000 population per year and leads to the demise of 30–50% of affected patients, principally because of sepsis or multiple organ dysfunction.

ARDS is an inflammatory disease characterized by an imbalance between pro- and anti-inflammatory compounds such as cytokines, and abnormalities of the coagulation system. Its pathology comprises hyaline membranes, endothelial and epithelial injury, loss of epithelial integrity, and increased alveolar-capillary permeability resulting in diffuse alveolar damage, with neutrophils, macrophages, erythrocytes, hyaline membranes, and protein-rich edema fluid in the alveolar spaces.

This loss of alveolo-capillary integrity, increases fluid flux into the alveoli, and thereby causes the clinical manifestations of ARDS. In addition, alveolo-capillary barrier leakiness can also lead to loss of lung compartmentalization, with the result that inflammatory mediators from the lung can enter the circulation and induce systemic consequences (multiple organ dysfunction).

After the acute phase of acute lung injury and the acute respiratory distress syndrome, some patients have an uncomplicated course with rapid resolution of the disorder. Other patients show progression to fibrotic lung injury which can be observed histologically as early as five to seven days after the onset of the disorder. The alveolar space becomes filled with mesenchymal cells and their products, along with new blood vessels.

The underlying mechanisms leading either to resolution of the inflammatory-cell infiltrate or fibrosis are unclear. Apoptosis (programmed cell death) is thought to be a major mechanism for the clearance of neutrophils from sites of inflammation and may be important for the clearance of neutrophils from the injured lung (31).

ROLE OF HEAT SHOCK PROTEINS AS POTENTIAL PHYSIOPATHOLOGICAL ACTORS AND THERAPEUTIC TARGETS

The treatment of ARDS is merely supportive because the patho-physiology of this highly lethal disease is poorly understood. Recognition of some key components of ARDS such as inflammation, epithelial dysfunction, apoptosis and fibrosis, prompted interest in the role of heat shock proteins as potential physiopathological actors and possible therapeutic targets. The evidence is as follows:

During acute lung injury several non-thermal inducers of stress proteins such as oxidant injury, inflammation, and ischemia-reperfusion are present. Moreover, recent data show that HSP-70 can limit the inflammatory response, protect proteins from damage, restore function of proteins that are damaged, and prevent cell destruction in lung tissues. Several examples of stress protein-mediated cytoprotection exist in cell and animal models of acute lung injury (23, 32, 40).

IN VITRO STUDIES

In vitro studies indicate that several mechanisms may account for the favorable effects of HSP in the lung. Recent *in vitro* studies have demonstrated that in pulmonary cells, cytoprotective effects of HSP may involve attenuation of endotoxin-mediated apoptosis and/or antioxidant effects (35).

Alternatively, by binding to cytokines and preventing their release from inflammatory cells, HSPs also have anti-inflammatory effects. In the cultured human respiratory epithelium, induction of the stress response inhibited tumor necrosis factor-alpha and prointerleukin-1B gene expression (40).

HSP70 binds intracellular tumor necrosis factor-alpha and prevents its release from the cells, an effect that has been suggested to be mediated by NF-kB. Indeed, HSP70 overexpression by plasmid-mediated gene transfer inhibits nuclear factor-kB (NF-kB) nuclear translocation (39).

Another important aspect of the stress response-mediated protection by HSP is related to inhibition of iNOS gene expression. In cultured rat pulmonary artery smooth muscle cells and murine respiratory epithelium, the stress response inhibits cytokine-mediated iNOS gene expression without affecting cell viability (37, 38).

Interestingly in cultured pulmonary cells the stress-induced suppression of proinflammatory gene expression appears to be selective, not generalized because surfactant protein expression is preserved (36).

IN VIVO STUDIES

Consistent with these positive results *in vitro*, studies *in vivo* and *ex-vivo* animal models have shown protective effects of HSP in experimental acute lung injury. Villar *et al.* were the first to demonstrate a cytoprotective effect of stress protein induction in a rat model of

acute lung injury caused by intratracheal administration of phospholipase A1. HSP70 was induced in the lungs of experimental animals by subjecting them to whole body hyperthermia (41C for 15 minutes) 18 hours before phospholipase administration. Heat-treated animals were significantly resistant to phospholipase A1-mediated acute lung injury, and had decreased mortality at 48 hours compared with control (non-heated) animals (28). Using the same heat preconditioning model, it was subsequently demonstrated that stress protein induction also protected against lung injury caused by intratracheal administration of TNF-alpha or systemic administration of endotoxin (30). More importantly, the whole body heating-induced stress response also had protective effects against acute lung injury when initiated after an endotoxin challenge (17). In these models, increased survival was correlated with blunted endotoxin-mediated iNOS mRNA expression in the lung, significant reduction of peak plasma concentration of cytokines (in particular IL-1-beta), attenuated neutrophil recruitment (11), and decreased microvascular protein permeability (5).

Similar positive results were obtained in another experimental model of lung injury: the ventilator-induced acute lung injury. Following mechanical ventilation with high tidal volume, heat preconditioned lungs had smaller decrease in lung compliance, lower plasma cytokine levels (TNF-alpha, Interleuline-1 beta, macrophage inflammatory protein-2) and an increased amount of active surfactant aggregate in BAL, compared to lungs from non-preconditioned animals (16, 29).

Taken together, although the mechanism of protection or the involvement of specific stress proteins remain incompletely understood, these studies suggest that stress protein induction could represent a novel therapeutic strategy for acute lung injury. However, an important limitation of these studies was that the stress response was produced by heating the animals or by using sodium arsenite, approaches that are not readily amenable to clinical application. Moreover, these treatments caused a full systemic stress response, and did therefore not reveal the underlying mechanism of protection in a given organ system.

To overcome some of these limitations, Weiss and colleagues tested the hypothesis that direct intratracheal adenoviral-mediated overexpression of the HSP-70 would improve the outcome of acute lung injury secondary to cecal ligation and perforation (a standard model for producing sepsis and a subsequent ARDS-like syndrome) in mice *in vivo*. The results were impressive: 48 hour mortality was cut in half, and edema and neutrophil accumulation in the alveolar space of treated mice were significantly attenuated (32). Consistent with these observations, adenovirus-mediated transfer of the stress protein heme oxygenase-1 cDNA into the lungs attenuates the severity of lung injury induced by the influenza virus in mice (9).

In summary, these data in experimental animals suggest that the stress response has selective inhibitory effects on the expression of genes relevant to lung injury and function. The mechanisms by which the stress response protects against ALI may involve selective inhibition of potentially deleterious patterns of gene expression (i.e. iNOS, TNF-a, and other NF-kB-mediated inflammatory processes) while allowing ongoing expression of beneficial patterns of gene expression (i.e. surfactant protein). Finally, selective adenovirus-mediated overexpression of stress proteins in the mouse augments survival after acute lung injury.

HEAT SHOCK PROTEINS, CHAPERONES AND RESPIRATORY TRANSEPITHELIAL ION TRANSPORT IN ACUTE LUNG INJURY

Because epithelial injury contributes to pulmonary edema by facilitating alveolar flooding and disrupting normal transepithelial ion and alveolar fluid clearance mechanisms, the degree of alveolar epithelial injury is an important predictor of the outcome of ARDS. Strategies that hasten the resolution of pulmonary edema may therefore be as important as those that attenuate early inflammatory lung injury, as suggested by the observation showing that maintenance of the ability to remove alveolar fluid is associated with improved oxygenation, a shorter duration of mechanical ventilation, and an increased likelihood of survival (12, 31).

Pulmonary edema results from a persistent imbalance between forces driving fluid into the airspaces and biological mechanisms for its removal. There is abundant evidence that active ion transport across the alveolar epithelium creates an osmotic gradient that leads to alveolar fluid clearance both during the perinatal period and in the adult lung. Sodium enters the apical membranes of alveolar epithelial cells through amiloride-sensitive cation channels, such as the epithelial sodium channels (ENaC) and the non-selective cation channels, and is then transported across the basolateral membrane into the interstitium by the ouabain-inhibitable Na-K-ATPase. ENaC is thought to be the limiting factor regulating transepithelial sodium transport and alveolar fluid clearance in the lung, because even a small fraction of the normal Na-K-ATPase activity appears to be sufficient to maintain normal ion transport (22).

In humans, indirect evidence suggests that a possibly genetic and /or acquired (see next paragraph) impairment of transepithelial sodium and water transport predisposes to high-altitude pulmonary edema (HAPE) (21), and plays a role in the pathogenesis of the RDS of the newborn (2).

Recently, increased transporter movement from putative intracytoplasmic pools to the cell membrane (intracellular trafficking) and stability of the transporter at the cell membrane has been suggested to stimulate ion transport (19, 25), but in particular with regard to ENaC this possibility is not proved. A defect in protein processing of membrane transporters has been shown to play a role in human disease such as cystic fibrosis (inefficient folding of the chloride channel CFTR) and Liddle's syndrome (increased stability of the ENaC at the cell membrane) (19).

In the kidney only a few percent (1-5%) of the ENaC synthesized in the endoplasmic reticulum reaches the cell surface (Figure 3). This may be due to rapid destruction of ENaC, related to incomplete protein folding and rapid channel degradation by endocytosis and ubiquitination (19).

It is well established that specific disease-related factors (for example: hypoxia/ hypoxemia, nitric oxide, cytokines, reactive oxygen species or pro-apoptotic molecules) downregulate sodium and water transport across the alveolar epithelium, and thereby impair alveolar fluid removal and favor pulmonary edema (22). The underlying mechanisms are still poorly understood, but, as recently shown for hypoxia, may involve dysregulation of ENaC processing and stability to the membrane (15) (Figure 3).

These observations could be consistent with the hypothesis that a genetic and/or ac-

quired defect of ENaC processing in the lung may augment the susceptibility to pulmonary edema, whereas increased efficiency of this processing may prevent alveolar fluid flooding during lung injury. This has led to studies examining the effects of interventions aimed to augment protein processing, such as stress-preconditioning or chemical chaperones, on respiratory transepithelial ion and water transport.

Upregulation of the heat shock protein 70 has been shown to stimulate intracellular processing of the chloride channel CFTR and partially restore its function in cells with a genetic defect of the intracellular processing of this channel (which has been shown to interact with the ENaC to regulate the respiratory transepithelial ion transport) (7). More importantly, administration of chemical compounds having chaperone activities similar to those characteristic for heat shock proteins, increased CFTR membrane expression and transepithelial chloride transport not only in mice with a genetic defect of CFTR processing, but also in their wild-type littermates (8) (Figure 4). Finally, during ischemia/reperfusion-induced lung injury, stress proteins allow to restore the ion and fluid transport capacity of the alveolar epithelium by upregulating alveolar fluid clearance in response to catecholamines (14) (Figure 5).

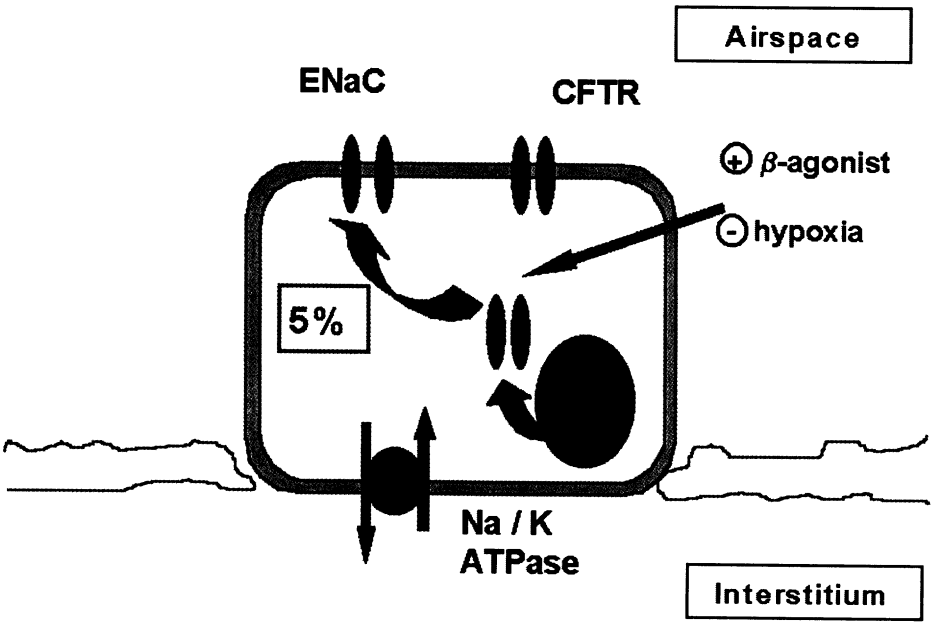

Figure 3. Only a few percent (1-5%) of the ENaC synthesized in the endoplasmic reticulum reaches the cell surface. It was recently suggested that the intracellular processing of the ENaC may be modulated by external factors such as drugs or disease-related factors. Whether endogenous (HSPs) or exogenous (chemical chaperones) may also influence apical ion channels processing, and in turn transepithelial sodium transport in alveolar type II cells is currently under investigation.

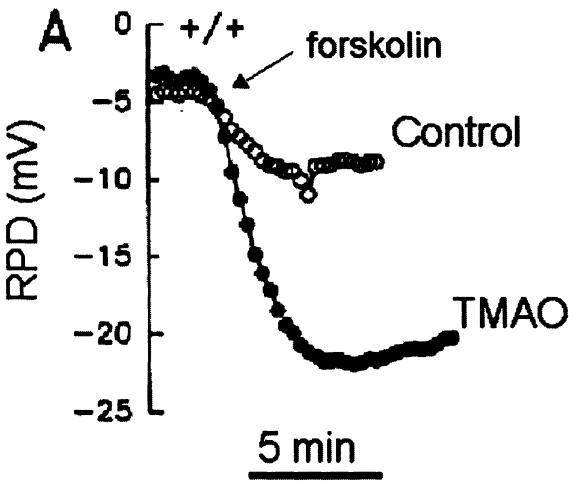

Figure 4. Effect of the chemical chapterone TMAO on chloride transport in the rectum of mice. The chemical chaperone trimethyl amino oxide (TMAO) increases the forskolin-dependent rectal potential difference (RPD) in wild type mice. This suggests that chemical chaperones may represent a novel therapy to augment ion channels expression at the cell membrane and transepithelial ion transport (8).

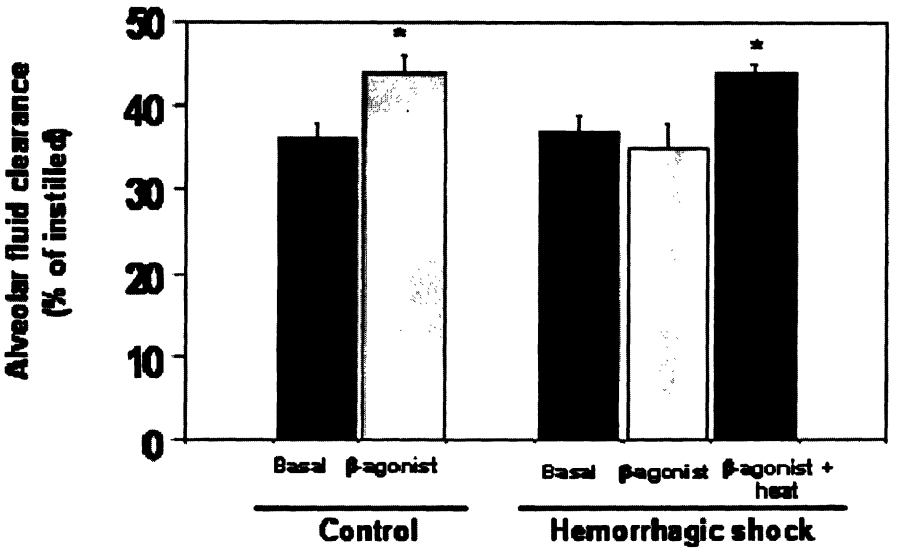

Figure 5. Effects of heat preconditioning on basal and ß-agonist-stimulated alveolar fluid clearance in rate after haemorrhagic shock Hemorrhagic shock abolishes the beta-agonist-mediated stimulation of alveolar fluid clearance in rats. Heat preconditioning reestablishes the normal ability of beta-agonists to stimulate transepithelial sodium and water transport (adapted from 14).

CLINICAL STUDIES

In contrast to cardiology (4, 24), clinical studies examining the role of heat shock proteins and/or chemical chaperones in the patho-physiology of pulmonary diseases are very sparse. In one study, alveolar macrophages from patients with ARDS spontaneously expressed large amounts of HSP70, suggesting a link between stress proteins and lung inflammation in humans (10).

More recently, 4 - phenylbutyric acid (PBA, a low-molecular weight fatty acid) has been shown to have chaperone-like activities, and when administered in patients with cystic fibrosis, PBA improved the apical surface CFTR function, as evidenced by a small but significant increase in nasal potential difference (20, 41). No data exist so far, concerning the possible role of PBA in the treatment of pulmonary diseases associated with impaired alveolar fluid clearance.

HSPs IN PATIENTS SUFFERING FROM SEVERE TRAUMA

Although typically regarded as intracellular proteins, it has recently been reported that heat shock proteins are released from cultured cells. For example, HSP60 and HSP72 have been detected in the plasma of healthy human subjects. In addition to being involved in the modulation of the immune system or serve as antigen carriers for antigen presenting cells, circulating HSPs could also represent a marker of the degree of stress experienced by the organism. Alternatively, circulating HSPs may indicate the ability of the stressed organism to conveniently respond to such a stress.

Consistent with the latter hypothesis, Hsp 72 can be detected in the serum of severely traumatized patients within 30 minutes after injury, and high initial serum levels of Hsp 72 (> 15 ng/mL) were associated with improved survival (13). This could suggest that either trauma survivors have an increased ability to respond to stress, and/or that increased HSP expression may confer protection against severe trauma and its complications.

SUMMARY AND FUTURE DIRECTIONS

Forty years after Ritossa's observation in the Drosophila fly that cells respond to stress by increasing the expression of genes coding for a certain class of cytoprotective proteins, it is know well established that this stress response plays an important role in cardiovascular diseases in humans. Recent data suggest that HSPs may also exert protective effects in the lung.

Preliminary data suggest that chemical chaperones may be useful for the treatment of cystic fibrosis. More importantly, the stress response markedly decreases mortality rates and attenuates cellular insults in several models of acute lung injury and sepsis, suggesting that in the near future induction of the stress response may represent a novel therapeutic tool also for the prevention and/or treatment of pulmonary edema associated with ARDS or heart failure.

REFERENCES

1. Ashbaugh DG and Petty TL. Sepsis complicating the acute respiratory distress syndrome. *Surg-GynecolObstet* 135: 865-869, 1972.
2. Barker PM, Gowen CW, Lawson EE, and Knowles MR. Decreased sodium ion absorption across nasal epithelium of very premature infants with respiratory distress syndrome [see comments]. *J Pediatr* 130: 373-377, 1997.
3. Benjamin IJ. Stress proteins: is their application in clinical medicine on the horizon? *Hepatology* 18: 1532-1534, 1993.
4. Benjamin IJ and McMillan DR. Stress (heat shock) proteins: molecular chaperones in cardiovascular biology and disease. *Circ Res* 83: 117-132, 1998.
5. Chen SC, Lu TS, Lee HL, and Lue SI. Hyperthermic pretreatment decreases microvascular protein leakage and attenuates hzpotension in anaphylactic shock in rats. *Microvascular Research* 61: 152-159, 2001.
6. Christou HM, T; Hsieh, CM; Koike, H; Arkonac, B; Perrella, MA; Kourembanas, S. Prevention of hypoxia-induced pulmonary hypertension by enhancement of endogenous heme oxygenase-1 in the rat. *Circulation Research* 86: 1224-1229, 2000.
7. Fang X, Fukuda N, Barbry P, Sartori C, Verkman AS, and Matthay MA. Novel role for CFTR in fluid absorption from the distal airspaces of the lung. *J Gen Physiol* 119: 199-207, 2002.
8. Fischer H, Fukuda N, Barbry P, Illek B, Sartori C, and Matthay MA. Partial restoration of defective chloride conductance in DeltaF508 CF mice by trimethylamine oxide. *Am J Physiol Lung Cell MolPhysiol* 281: L52-L57, 2001.
9. Hashiba T, Suzuki M, Nagashima Y, Suzuki S, Inoue S, Tsuburai T, Matsuse T, and Ishigatubo Y. Adenovirus-mediated transfer of heme oxygenase-1 cDNA attenuates severe lung injury induced by the influenza virus in mice. *Gene Ther* 8: 1499-1507, 2001.
10. Kindas-Mugge I, Pohl WR, Zavadova E, Kohn AD, Fitzal S, Kummer F, and Micksche M. Alveolar macrophages of patiet with adult respiratory distress syndrome express high levels of heat shock protein 72 mRNA. *Shock* 5: 184-189, 1996.
11. Koh Y, Lim CM, Kim MJ, Shim TS, Lee SD, Kim WS, Kim DS, and Kim WD. Heat shock response decreases endotoxin-induced acute lung injury in rats. *Respirology* 4: 325-330, 1999.
12. Matthay MA and Wiener-Kronish JP. Intact epithelial barrier function is critical for the resolution of alveolar edema in humans. *Am Rev Respir Dis* 142: 1250-1257, 1990.
13. Pittet JF, Lee H, Morabito D, Howard MB, Welch WJ, and Mackersie RC. Serum levels of Hsp 72 measured early after trauma correlate with survival. *J Trauma* 52: 611-617; discussion 617, 2002.
14. Pittet JF, Lu LN, Geiser T, Lee H, Matthay MA, and Welch WJ. Stress preconditioning attenuates oxidative injury to the alveolar epithelium of the lung following haemorrhage in rats. *J Physiol* 538: 583-597, 2002.
15. Planes C, Blot-Chabaud M, Matthay MA, Couette S, Uchida T, and Clerici C. Hypoxia and beta2-agonists regulate cell surface expression of epithelial sodium channel in native alveolar epithelial cells. *J Biol Chem* 277: 47318-47324, 2002.
16. Ribeiro SP, Rhee K, Tremblay L, Veldhuizen R, Lewis JF, and Slutsky AS. Heat stress attenuates ventilator-induced lung dysfunction in an *ex vivo* rat lung model. *Am J Respir Crit Care Med* 163: 1451-1456, 2001.
17. Ribeiro SP, Villar J, Downey GP, Edelson JD, and Slutsky AS. Effects of the stress response in septic rats and LPS-stimulated alveolar macrophages: evidence for TNF-alpha posttranslational regulation. *Am J Respir Crit Care Med* 154: 1843-1850, 1996.
18. Ritossa F. A new puffing pattern induced by a temperature shock and DNP in Drosophila. *Experientia* 18: 571-573, 1962.
19. Rotin D, Kanelis V, and Schild L. Trafficking and cell surface stability of ENaC. *Am J Physiol Renal Physiol* 281: F391-F399, 2001.

20. Rubenstein RC and Zeitlin PL. A pilot clinical trial of oral sodium 4-phenylbutyrate (Buphenyl) in DF508/homozygous cystic fibrosis patients. *Am J Respir Crit Care Med* 157: 484-490, 1998.
21. Sartori C, Allemann Y, Duplain H, Lepori M, Egli M, Lipp E, Hutter D, Turini P, Hugli O, Cook S, Nicod P, and Scherrer U. Salmeterol for the prevention of high-altitude pulmonary edema. *NEnglJ Med* 346: 1631-1636, 2002.
22. Sartori C and Matthay MA. Alveolar epithelial fluid transport in acute lung injury: new insights. *Eur Respir J*: 1299-1313, 2001.
23. Slutsky AS. Hot new therapy for sepsis and the acute respiratory distress syndrome. *J Clin Invest* 110: 737-739, 2002.
24. Snoeckx L, Cornelussen R, Nieuwenhoven F, Reneman R, and Van der Vusse G. Heat schock proteins and cardiovascular pathophysiology. *Physiological Review* 81: 1461-1497, 2001.
25. Snyder PM. Liddle's syndrome mutations disrupt cAMP-mediated translocation of the epithelial Na(+) channel to the cell surface. *J ClinInvest* 105: 45-53, 2000.
26. Tissieres AM, HC; Tracy, UM. Protein synthesis in salivary glands of Drosophila melanogaster. relation to chromosomal puffs. *Journal of Molecular Biology* 84, 1974.
27. Villar J. Heat shock protein gene expression and survival in critical illness. *Crit Care* 4: 2-5, 2000.
28. Villar J, Edelson JD, Post M, Mullen JB, and Slutsky AS. Induction of heat stress proteins is associated with decreased mortality in an animal model of acute lung injury. *Am Rev Respir Dis* 147: 177-181, 1993.
29. Villar J and Mendez-Alvarez S. Heat shock proteins and ventilator-induced lung injury. *Curr Opin Crit Care* 9: 9-14, 2003.
30. Villar J, Ribeiro SP, Mullen JB, Kuliszewski M, Post M, and Slutsky AS. Induction of the heat shock response reduces mortality rate and organ damage in a sepsis-induced acute lung injury model. *Crit Care Med* 22: 914-921, 1994.
31. Ware LB and Matthay MA. The acute respiratory distress syndrome. *NEnglJMed* 342: 1334-1349, 2000.
32. Weiss YG, Maloyan A, Tazelaar J, Raj N, and Deutschman CS. Adenoviral transfer of HSP-70 into pulmonary epithelium ameliorates experimental acute respiratory distress syndrome. *J Clin Invest* 110: 801-806, 2002.
33. Welch WJ. How cells respond to stress. *SciAm* 268: 56-64, 1993.
34. Welch WJ. Mammalian stress response: cell physiology, structure/function of stress proteins, and implications for medicine and disease. *Physiol Rev* 72: 1063-1081, 1992.
35. Wong HR, Menendez IY, Ryan MA, Denenberg AG, and Wispe JR. Increased expression of heat shock protein-70 protects A549 cells against hyperoxia. *Am J Physiol* 275: L836-841, 1998.
36. Wong HR, Ryan M, Gebb S, and Wispe JR. Selective and transient *in vitro* effects of heat shock on alveolar type II cell gene expression. *Am J Physiol* 272: L132-138, 1997.
37. Wong HR, Ryan M, Menendez IY, Denenberg A, and Wispe JR. Heat shock protein induction protects human respiratory epithelium against nitric oxide-mediated cytotoxicity. *Shock* 8: 213-218, 1997.
38. Wong HR, Ryan M, and Wispe JR. The heat shock response inhibits inducible nitric oxide synthase gene expression by blocking I kappa-B degradation and NF-kappa B nuclear translocation. *Biochem Biophys Res Commun* 231: 257-263, 1997.
39. Wong HR, Ryan M, and Wispe JR. Stress response decreases NF-kappaB nuclear translocation and increases I-kappaBalpha expression in A549 cells. *J Clin Invest* 99: 2423-2428, 1997.
40. Wong HR and Wispe JR. The stress response and the lung. *Am J Physiol* 273: L1-9, 1997.
41. Zeitlin PL, Diener-West M, Rubenstein RC, Boyle MP, Lee CKK, and Brass/Ernst L. Evidence of CFTR function in cystic fibrosis after systemic administration of 4-phenylbutyrate. *Molecular Therapy* 6: 119-126, 2001.