Implications of heat shock/stress proteins for medicine and disease

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Abstract: Heat shock/stress proteins (HSPs) are crucial for maintenance of cellular homeostasis during normal cell growth and for survival during and after various cellular stresses. The HSP70 family functions as molecular chaperones and reduces stress-induced denaturation and aggregation of intracellular proteins. In addition to the chaperoning activities, HSP70 has been suggested to exert its protective action by protecting mitochondria and by interfering with the stress-induced apoptotic program. The biochemical and functional properties of HSPs observed in cultured cells may be relevant to organs and tissues in whole animals. The activation of the hypothalamic-pituitary-adrenal axis and the sympathetic nerve system elicits the stress response in selected peripheral tissues; the HSP70 expression in the vasculature and stomach increases resistance against hemodynamic stress and stress-induced mucosal damage, respectively. Gastric mucosa pretreated with mild irritants acquires a tolerance against subsequent mucosal-damaging insults. This phenomenon is known as "adaptive cytoprotection". Transient ischemia also induces ischemic tolerance in the brain and heart, which is called "ischemic preconditioning". The heat shock response is believed to contribute to the acquisition of the tolerance. The therapeutic applications of chaperone inducers that induce HSPs without any toxic effect are also introduced. J. Med. Invest. 44 : 137-147, 1998

Key Words : *heat shock/stress proteins, physiologic stress, stress ulcer, ischemic tolerance, chaperone inducers*

INTRODUCTION

The heat shock response, first observed in *Drosophila melanogaster* over thirty years ago (1), is now recognized to represent a universally conserved cellular defense program. The heat shock response is mediated by the increased expression of genes encoding a group of proteins referred to as the heat shock proteins (HSPs) or stress proteins. Over the last 30 years, the heat shock response has been observed in cells from all organisms; from bacteria to human. In addition to heat shock, a variety of metabolic insults, including heavy metals, amino acid analogs, oxidants, and different metabolic poisons, also elicits the response. Stress proteins are highly conserved with respect to their primary structure, mode of regulation, and biochemical function (2, 3). HSP expression is not limited to cells undergoing acute stress, and several members of HSP families are constitutively expressed. Many stress proteins maintain cellular homeostasis by acting as molecular chaperones (4-6). Molecular chaperones have been defined as proteins that bind to and stabilize an otherwise unstable conformer of another protein. By controlling binding and release, they participate in the folding and assemby of nascent and unfolded peptides and facilitate protein transport to a particular subcellular compartment and disposal by degradation (7). Stress proteins are classified into families according to their apparent molecular weights and respective inducers. Major stress proteins expressed in mammalian cells are listed in Table 1.

Stress proteins are crucial for the maintenance of cell integrity during normal cell growth as well as during pathophysiological conditions. Most of our knowledge concerning the homeostatic role of stress proteins has come from studies using cultured cells. The best example of the acquisition of tolerance by stress proteins is illustrated by the phenomenon of " acquired thermotolerance ". Cells subjected to a sublethal heat shock treatment or other insults, if they are provided an appropriate recovery period, are able to survive a second lethal stressor. Although much less is known about their expression in vivo, HSPs are acutely induced in intact animals in response to various metabolic insults, such as ischemia/reperfusion

Received for publication December 19, 1997 ; accepted January 8, 1998.

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Table 1. Major stress proteins expressed in mammalian cells

Name	Size (kDa)	Location	Remarks
ORP150	150	Endoplasmic reticulum	Hypoxia inducible
HSP 104/110	104/110	Cytosol/nucleus	Required to survive severe stress molecular chaperone (?)
HSP90	90	Cytosol/nucleus	Part of steroid hormone receptor complex ; chaperon for protein kinases (?)
Grp78 (Bip)	78	Endoplasmic reticulum	Constitutively expressed molecular chaperone
Grp75	75	Mitochondria	Constitutively expressed molecular chaperone
HSC70	73	Cytosol/nucleus	Constitutively expressed molecular chaperone
HSP70	72	Cytosol/nucleus	Highly stress inducible
HSP60	60	Mitochondria	Molecular chaperone (chaperonin)
TCP-1	60	Cytosol	chaperonin related to HSP60
HSP56	56	Cytosol	Part of steroid hormone receptor ; binds FK506
HSP47	47	Endoplasmic reticulum	Collagen chaperone
HSP40 (Hdj 1)	40	Cytosol/nucleus	Cofactor for HSP70
Small HSPs	20-30	Cytosol/nucleus	Proposed regulator of actin ; proposed molecular chaperone
HSP10	10	Mitochondria	Cofactor for HSP60
Ubiquitin	8	Cytosol/nucleus	Involved in protein degradation by proteasome

or inflammation, as well as whole body hyperthermia (8-10). Are the biochemical and functional properties of the heat shock response/proteins observed in cultured cells relevant to organs and tissues in the whole animal? In order to address this issue, in this review, we will focus on the HSP expression in vivo and on the clinical implications of the heat shock response/stress proteins. Because of space limitations, we will not describe the structure of stress proteins. In this regard, please refer to the recent reviews and references therein (2-7).

INDUCTION OF HEAT SHOCK RESPONSE IN VITRO

The expression of stress proteins is not only induced by elevated temperature, but also by several environmental stresses described above. Many of these agents/treatments share the common property of affecting the proper conformation of proteins. Consequently, the intracellular accumulation of unfolded or misfolded“ abnormal ”protein may be a common signal (11), but other mediators, including classical second messengers, such as intracellular free calcium, protein kinases, or alterations in DNA, have also been suggested to induce stress proteins (12, 13).

The stress response in mammalian cells is usually considered to be transcriptionally regulated by the activation of a pre-existing pool of the heat shock transcription factor (HSF), which binds to the heat shock promoter element (HSE) that is composed of at least three pentanucleotide modules (nGAAn) arranged as a contiguous inverted repeat (14). The HSF family includes HSF1, HSF2, HSF3, and HSF4 in higher eukaryotes (15-19). HSF1 is identified as the mediator of stress-induced transcription of heat shock genes (17, 20, 21). HSF2 has been suggested to be important for controlling the activities of heat shock gene expression in normal or unstressed cells (21). The precise physiological roles of HSF 3 and HSF 4 are not completely elucidated (18, 19).

HSF1 is present in normal, unstressed cells as a monomer. HSFs have two highly conserved regions: an NH_2-terminal DNA-binding domain of ~100 amino acids and an adjacent trimerization domain containing three leucine zippers. In higher eukaryotes, there is a fourth leucine zipper domain near the COOH-terminus that appears to interact directly

with the more NH_2-terminal leucine zipper array to prevent trimerization and to mask the nuclear localization signal in resting cells (22). As illustrated in Fig.1, upon exposure to stress, it rapidly trimerizes, acquires DNA-binding activity, is transported into the nucleus, and becomes transcriptionally competent (23, 24). It has been suggested that the acquisition of DNA-binding activity by HSF1 is independent of inducible phosphorylation, but acquisition of transcriptional activation is linked to inducible serine phosphorylation (25). The redox regulation is also suggested to be involved in the transcriptional activation of heat shock genes (26, 27).

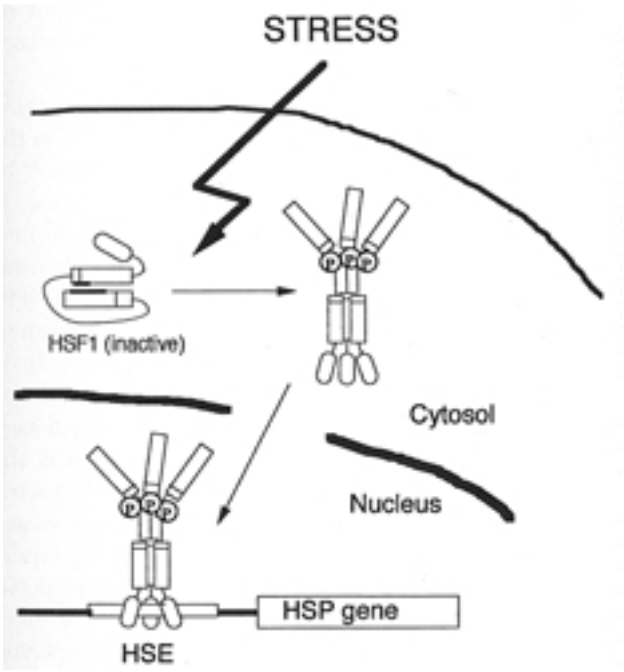

Fig.1. Model of HSF1activation. In resting cells, HSF1is present in the cytosol as a monomer. Stress induces trimerization, acquisition of HSF1-DNA binding activity, and nuclear translocation. Stress-inducible serine phosphorylation is required for transcriptional activation.

HOW STRESS PROTEINS PROTECT CELLS AGAINST DAMAGE UNDER STRESSFUL CONDITIONS

Members of the HSP70 protein family include: HSC70 (a constitutive HSP70), present within the cytoplasm and nucleus; grp75, mitochondrial HSP70; grp78(Bip), a resident of the endoplasmic reticulum. In addition, under conditions of stress, another form of the highly stress-inducible HSP70 (simply referred to here as HSP70) is synthesized at high levels. This stress-inducible HSP70 plays a critical role in the induction of resistance to various metabolic insults (28, 29). The HSP70 protein family functions as molecular chaperones in refolding of denatured polypeptide (4-7). In fact, overproduction of HSP70 was shown to reduce stress-induced denaturation and aggregation of certain proteins (30, 31), leading to the common assumption that refolding and antiaggregating activities of HSP70 determine its role in protection against stresses (32, 33). However, under some conditions, the protective action of HSP70 appears to be unrelated to its chaperoning action. TNF-α-induced apoptosis can be prevented by overexpression of HSP70 (34). This can be explained by the notion that overproduction of HSP70 interferes with the apoptotic program by suppressing the activation of JNK (35-38). Thus, the protective action of HSP70 in some circumstances may, at least in part, involve direct interference with the apoptotic program, although the molecular basis of this action is still unknown.

There is growing evidence that HSPs play an essential role in protecting cells against oxidative injury (39). Oxidative injury participates in a variety of pathological conditions, such as inflammation and ischemia/reperfusion injury. During inflammation, oxygen free radicals are generated by the phagocytic cells (polymorphonuclear leukocytes, monocytes-macrophages) infiltrating the inflamed tissues. Oxygen free radicals are also produced by a xanthine-xanthine oxidase system. Ischemia causes a decrease in ATP level related to uncoupling of oxidative phosphorylation, leading to the accumulation of xanthine and hypoxanthine. These substrates are normally metabolized by xanthine dehydrogenase. However, during ischemia and when the level of intracellular free calcium is elevated, the dehydrogenase reverts to xanthine oxidase. During reperfusion, xanthine and hypoxanthine are metabolized by xanthine oxidase, generating large amounts of superoxide anion. Oxygen free radicals are potent activators for HSP expression, and at the same time, overproduction of HSP70 protects cells against oxidative injury (39). For example, during activation, macrophages induce HSP70, to protect themselves against autooxidative damage associated with the enhanced respiratory burst activity (40).

Protective effects of HSP against oxygen radical-induced cellular damage may be targeted to any of the following: membranes (lipid peroxidation), proteins, DNA, and mitochondria. The protective effects of HSP70 against lipid peroxidation and DNA damage have been reviewed (41). Recently, Polla et al. suggested that mitochondria are selective targets for the protective effects of heat shock against oxidative injury (42). They demonstrated that overproduction of HSP70 by heat shock prevented hydrogen peroxide-induced decline of mitochondrial permeability transition and swelling of mitochondria, which are suggested to make the“ decision to die ”in the effector phase of the apoptotic process (43). Consequently, mitochondria may represent a key organella in the choice of necrosis (amplification of inflammation) or apoptosis (limitation of inflammation). Therefore, HSP70 may protect cells against oxidant-induced apoptosis. Thus, HSP overexpression may protect multiple cellular compartments and induce resistance of the cell against damage caused by various metabolic insults.

INDUCTION OF STRESS PROTEINS IN RESPONSE TO PHYSIOLOGICAL STRESS

The ability to preserve homeostasis under stressful conditions is a requisite for survival of all organisms in an everchanging environment. At the cellular level, the stress

response is well characterized to be mediated by the rapid expression of heat shock genes. However, relatively little information is available on HSP induction in vivo and on its roles in normal as well as pathological conditions. Holbrook and colleagues have demonstrated in the rat that expression of the major HSP, HSP70, is induced in vivo in response to a variety of stresses, including mild elevations in body temperature (>1.5°C), ether anesthesia, surgery, and restraint stress (8, 44-46). They found that the response was present in the adrenal gland and vasculature and absent in all other tissues examined (44, 46). Restraint causes the rapid expression of HSP70 mRNA with a peak at 30-60 min after starting the stress. The induction of HSP70 transcript is followed by an elevation in HSP70 protein, with maximum expression occurring between 3 and 6 hours after restraint (46).

The restraint-induced HSP70 expression is, at least in part, regulated by neuroendocrine mechanisms. Stress induces the secretion of corticotropin-releasing hormone (CRH) from the hypothalamus, which in turn results in secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. ACTH then stimulates the adrenal cortex, increasing both the synthesis and release of glucocorticoids into the peripheral circulation. CRF also activates the sympathetic nerve center in the brain stem, resulting in the synthesis and release of catecholamines from both peripheral ganglia and the adrenal medulla. Hypophysectomy abolished the response of the adrenal cortex, and the addition of ACTH restored specific expression in the hypophysectomized rats, suggesting that ACTH mediates the adrenal response (47).

In contrast to the adrenal response, elevated HSP70 mRNA was observed in the aorta of hypophysectomized animals after restraint regardless of the presence or absence of ACTH or dexamethasone. A specific α1 adrenergic-blocking agent, prazosin, virtually eliminated the induction of HSP70 in the vasculature, while the β adrenergic receptor antagonist, propranolol, had a lesser effect (46). Furthermore, the specific α1 adrenergic agonist, phenylephrine, induced the expression of HSP70 in the aorta, suggesting that the vascular response to restraint is dependent on activation of the sympathetic nervous system, especially via α1 adrenergic receptor (48). The physiological meaning of HSP induction in the vasculature is not completely understood. However, recent evidence suggests that the response plays an important role in protection of arteries against hemodynamic stress. Acute hypertension caused by treatment with various hypertensive agents, including phenylephrine, angiotensin II, and vasopressin, induces HSP gene expression in rat arterial wall (49, 50). Another interesting finding is that the rat strain with a genetic hypertensive background (SHR, spontaneously hypertensive rat) shows enhanced heat shock response in the aorta (51). Alternatively, overexpression of HSP70 prevents endotoxin-induced hypotension (52). Thus, HSP70 in the vasculature appears to induce resistance against hemodynamic stress.

STRESS PROTEINS IN THE STOMACH

The stomach is frequently exposed to hot food, ethanol, and oxidants generated from ingested food, cigarette smoke, and *Helicobacter pylori*-associated inflammation. Gastric surface epithelial cells are the first line of defense against these irritants. Primary cultures of gastric surface epithelial cells from guinea pig fundic glands exhibit a typical heat shock response (27, 53). In order to study the physiological roles of stress proteins in the stomach, we focused on the HSP induction in the stomach after exposure of rats to restraint and water-immersion stress. This stress causes severe ulceration in the stomach; therefore, it is an excellent model for revealing the importance of stress proteins in gastric mucosal cytoprotection.

Restraint and water-immersion stress caused rapid expression of HSP90, HSC70, and HSP70 mRNAs in the hypothalamus, and these expressions were followed by inductions of the respective HSP proteins. However, in this case, HSP90 was more remarkably induced than HSP70. When the stress-induced HSP90 expression was examined in various brain regions, the elevation of HSP90 induction was observed selectively in the hypothalamus, hippocampus, and amygdala, all of which participate in mediating stress responses (Fig.2). The restraint and water-immersion stress activates the hypothalamic-pituitary-adrenal axis, and HSP70 induction was observed in the adrenal gland. The stress rapidly activated HSF1 in gastric mucosa within 15 min, and HSP70 mRNA expression was detected with a peak at 30 min, followed by induction of HSP70 protein (Fig.3). The gastric mucosal response preceded the formation of gastric mucosal lesion, since macroscopic ulceration was first detected at 2 hours after

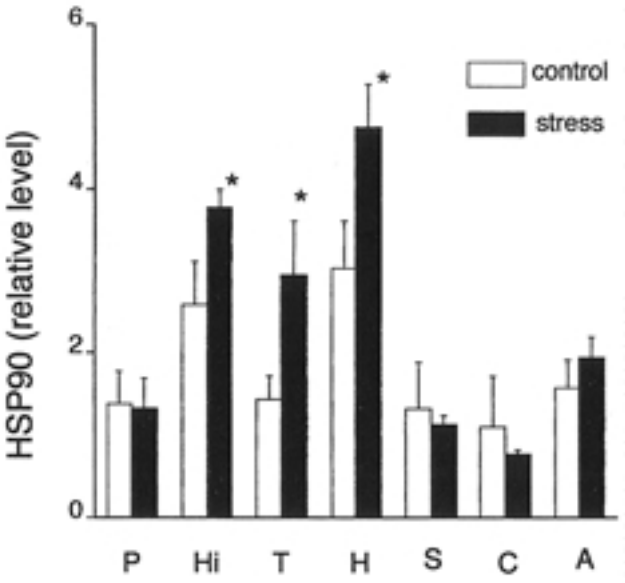

Fig.2. Accumulation of HSP 90 in rat brain regions after exposure to restraint and water-immersion stress. Before and after exposure of rats to restraint and water-immersion stress for 2 h, tissue proteins were extracted from A, amygdala ; Hi, hippocampus ; S, striatum ; T, thalamus ; H, hypothalamus ; C, cortex ; and P, pyriform cortex. The HSP level was measured by immunoblot analysis with a polyclonal anti-HSP90 antibody. Values are the mean±SD from 3 animals. *P < 0.05 by Student's t-test, compared with unstressed rats.

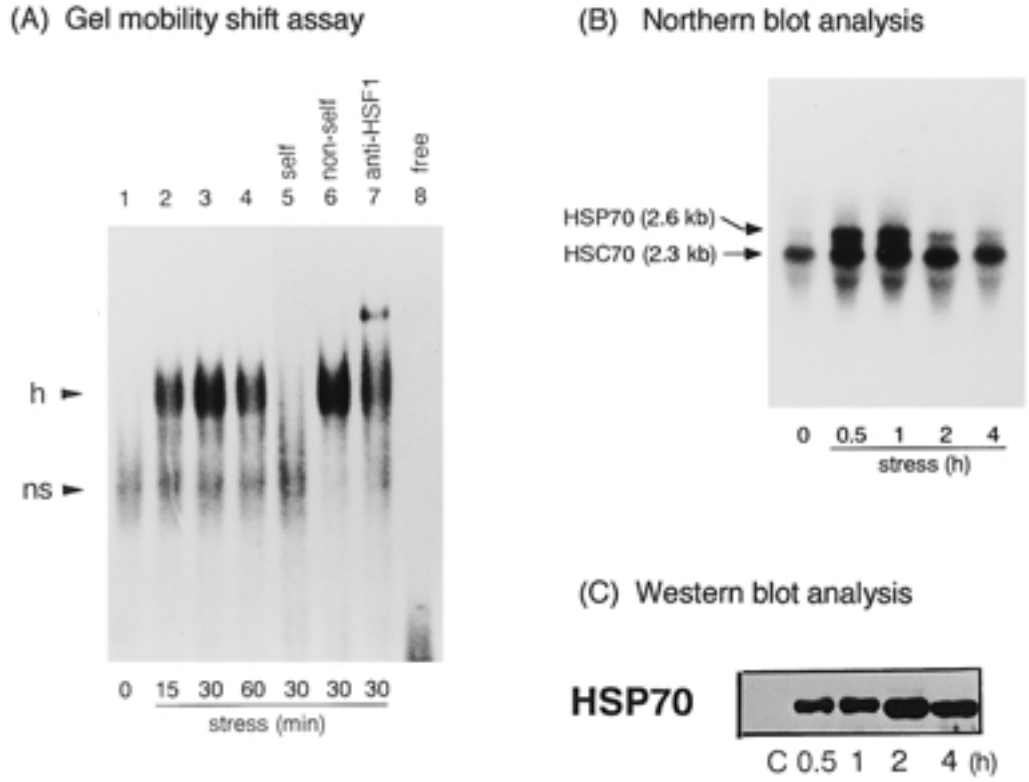

Fig.3. Activation of HSF1 and expression of HSP70 mRNA and protein in gastric mucosa of rats exposed to restraint and water-immersion stress. (A) Before and after exposure to restraint and water-immersion stress for the indicated times, total cellular protein was extracted from gastric mucosa, and gel mobility shift assay was performed with [^{32}P]HSE oligonucleotide. Lane 5 (marked self) and lane 6 (marked non-self) contained a 50-fold molar excess of unlabeled HSE oligonucleotide and the AP-1 oligonucleotide, respectively. Lane 7 shows the supershift experiment with an antibody against HSF 1. Interaction shown by" h "was specific HSE-binding activity." ns ", nonspecific interaction. (B) Total RNA was extracted from gastric mucosa of the rats and subjected to Northern hybridization with a cDNA probe for human HSP70. (C) The HSP70 protein level in the gastric mucosa was measured by immonoblot analysis with a polyclonal antibody against HSP70.

starting the stress.

In order to better understand the role of HSP expression in gastric mucosa, we exposed three experimental models; protein-malnourished rats, adrenalectomized rats, and vagotomized rats, to restraint and water-immersion stress (Fig.4). Rats fed a low-protein diet had a markedly reduced stress-induced HSP70 mRNA expression in the hypothalamus, adrenal gland, and stomach. The stress ulcer formation was enhanced in these animals. Although the HSP70 mRNA expression in the hypothalamus was rather enhanced in the adrenalectomized rats, bilateral adrenalectomy completely blocked the stress signal from the hypothalamus to the stomach, and the stress response was absent in the stomach, causing the most severe damage in the stomach. In contrast, subdiaphragmatic vagotomy almost completely prevented the stress ulcer formation. In this case, the HSP induction was markedly enhanced; HSP70 mRNA expression was acceralated and remained elevated for more than 4 hours (Fig.4). Thus, the extent of HSP induction was inversely correlated to the severity of gastric mucosal damage. We also found that the HSP expression in gastric mucosa was regulated by the activation of HSF1. These results strongly suggest that the gastric mucosal response is mediated by the activations of hypothalmic-pituitary-adrenal axis and sympathetic nerve system, and that HSPs, especially HSP70, induce resistance of gastric mucosa against stress-induced mucosal damage. Thus, HSPs play a fundamental protective role in gastric mucosa under stressful conditions.

STRESS PROTEINS IN THE CENTRAL NERVOUS SYSTEM AND HEART

Transient ischemia induces HSPs within certain regions of the brain, and it is of particular interest that the ability of a neuronal population to survive an ischemic trauma appeared to be correlated with increased expression of HSPs (9). The induction of the stress-inducible HSP70 after transient ischemia was most pronounced in the dentate granule cells and the hippocampal CA3 cells, where neuronal cells exhibit the highest survivability following the ischemic trauma. In contrast, HSP70 induction is minimal in those regions, like the hippocampal CA1 region, that appeared to be most sensitive to the ischemic episode (54). In addition to ischemia, stress protein induction has been observed in various pathological conditions such as trauma, epilepsy, elevated body temperature, neurodegenerative diseases, excitatory amino acids such as glutamate, and drug administration (for reviews see 55 and 56). Certain neuronal cells pretreated with mild heat

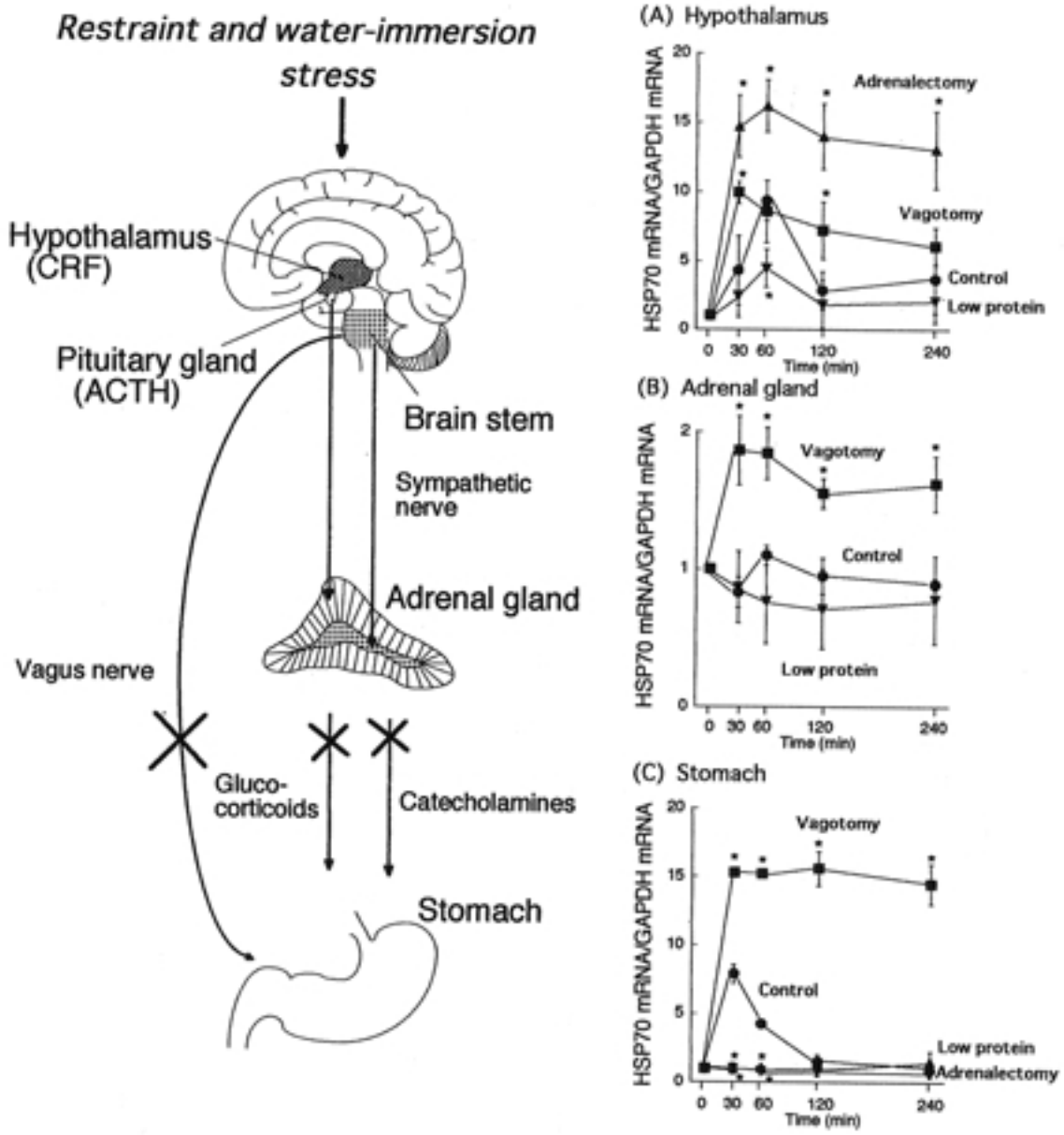

Fig.4. Expression of HSP70 mRNA in the hypothalamus, adrenal gland, and gastric mucosa of rats exposed to restraint and water-immersion stress. Control rats fed a 20% casein diet (●) or rats fed a 5% casein diet (▼) for 3 weeks were exposed to restraint and water-immersion stress. Rats fed a 20% casein diet received bilateral adrenalectomy (▲) or truncal vagotomy (■), and then they were enforced with the same stress one week after the operation. Before and after exposure to the stress for the indicated times, total RNA was extracted from the hypothalamus (A), adrenal gland (B), and gastric mucosa (C), and the HSP70 mRNA level was measured by Northern blot analysis, as described in the legend to Fig.3. The HSP70 mRNA level was quantified by densitometric analysis and standardized by the mRNA level of glyceraldehyde-3-phosphate dehydrogenase.

shock or sublethal ischemia acquire a tolerance against subsequent lethal ischemic stress. Stress proteins are believed to contribute to the acquisition of this tolerance (57-60). Recently, Kuwabara et al. identified a novel stress protein, the 150-kDa oxygen-regulated protein (ORP150), which is selectively induced in astrocytes exposed to hypoxia. This ORP is also expected to induce ischemic tolerance of astroglia (61).

In the heart, induction of stress response has been observed under physiological stresses, such as ischemia (10, 62, 63), trauma (64), hemodynamic overload (65, 66), and exercise (67), as well as hyperthermia (68). Induction of HSPs by pretreatment with heat shock or transient ischemia has been shown to be correlated with improvement of functional recovery (69-71) and reduction of infarct size (68, 72, 73). These protective roles were demonstrated in transgenic mouse overexpressing HSP70 in the heart (74-76).

In the brain and heart, the acquisition of ischemic tolerance, which is sometimes referred to as" ischemic preconditioning "is an attractive phenomenon for physicians. The factors that induce the tolerance would be potential targets for treatment and prevention of cerebrovascular diseases and myocardial infarction. Stress proteins are believed to play an important role in the ischemic preconditioning.

IMPLICATIONS OF CHAPERONE INDUCER FOR MEDICINE AND DISEASE

When cells are under sudden stress from heat, toxins, or disease-causing microorganisms, cellular proteins often lose their proper shape (i. e. aggregation), and HSP numbers quickly double (usually 10% of the protein mass of a cell). These HSPs rush to rescue the injured protein, repairing damage by binding to them and helping to fold them properly again (4-7). HSPs also bind to irreversibly damaged protein, helping to facilitate their degradation through the ubiquitin-proteasome pathway of proteolysis or lysosomal proteolysis (for reviews see 77 and 78). A large body of information supports that many HSPs work as molecular chaperones and are crucial for the maintenance of cell integrity during normal growth as well as during pathophysiological conditions. Therefore, it would be of great therapeutic benefit to discover compounds that induce HSPs without any toxic effect.

Biorex Research & Development Co., Hungary, has introduced a group of drugs in development that works by triggering the production of stress proteins. One hydroxylamine derivative (called Bimoclomol) that was originally developed to prevent microangiopathy in diabetes patients is now under Phase II clinical trials. Biorex is already testing similar drugs for stroke and atherosclerosis. Bimoclomol does not directly induce HSP70, but it amplifies the induction when cells are exposed to stressful conditions (79). There are numerous compounds that trigger the HSP induction; however, in most cases, they produce harmful conditions. We introduced a non-toxic chaperone inducer for the first time (80). Geranylgeranylacetone (GGA), an acyclic polyisoprenoid, is an antiulcer drug developed in Japan and has been widely used for more than 13 years. This drug rapidly induces resistance of gastric mucosal cells to irritants within 30 min in vivo and in vitro. We demonstrated that GGA can directly activate HSF1 and transiently cause transcriptional activation of heat shock protein genes to a lesser extent in both cultured gastric epithelial cells and rat gastric mucosa (80). This compound also enhances heat shock response of gastric mucosa of rats exposed to restraint and water-immersion stress and suppresses stress ulcer formation (Fig.5). GGA has been widely used as an antiulcer drug with a previously unrealized action that induces HSPs without any toxic effect. Nontoxic chaperone inducers may have potential therapeutic benefits for treatment and prevention of several diseases, such as ischemia/reperfusion injury, trauma, inflammation, infection, stress ulcer, and organ transplantation (Fig.6).

In addition to studies on the protective effects of stress proteins on ischemia/reperfusion injury in the brain and heart, there are several on-going projects that target stress proteins. For example, the capacity of HSPs as chaperones might prevent the accumulation of deadly plaques in neurodegenerative ailments such as Alzheimer s disease. Linquest has shown that stress proteins regulate another closely watched class of proteins, prions, which are prone to improper folding. Malformed prions is believed to cause

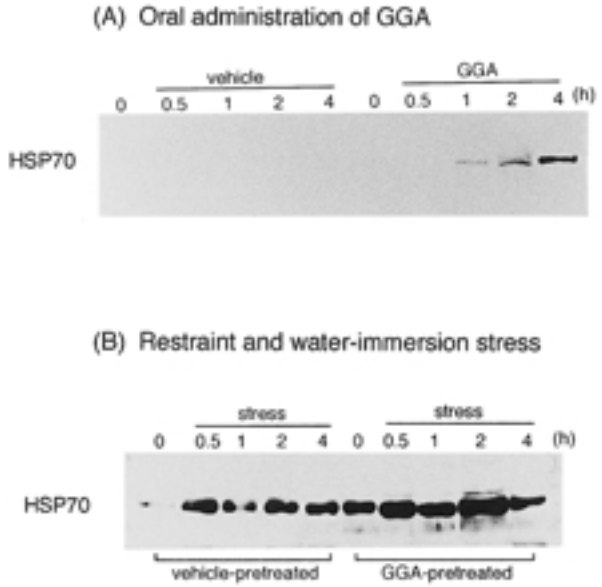

Fig.5. Effects of geranylgeranylacetone (GGA) on HSP70 induction in rat gastric mucosa. Gastric mucosa was collected from rats at the indicated times after intragastric administration of GGA or vehicle (A). Gastric mucosa was also isolated after exposing rats, pretreated with GGA or vehicle for 2 h, to restraint and water-immersion stress (B). Tissue proteins were extracted from gastric mucosa and subjected to immunoblot analysis with an antibody against HSP70.

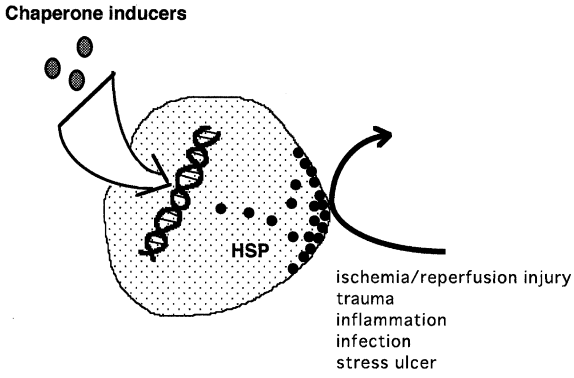

Fig.6. Therapeutic implications of chaperone inducers.

mad cow disease as well as human Creutzfeldt-Jakob disease (81).

Now immunologists are also using stress proteins to develop vaccines for AIDS and other infectious diseases and for treatment of cancer. Stress proteins themselves (HSP65 and HSP70) are potent stimuli of the immune system (for reviews see 82-84). The immune responses raised against pathogen HSPs appear to be essential in protective immunity. HSPs are highly conserved in all organisms and the molecular mimicry may lead to autoimmune reactions in the host (83). HSPs may participate in the processing and/or presentation of exogenous antigens. A possible involvement of HSPs in the antigen presentation is suggested by the structural similarities between major histocompatibility complex (MHC) class 1 and structural models of HSP70 (4). It has been suggested that tumor cells express HSP70 and HSP90 on the cell mem-

brane. HSC70 has been suggested to be a transformation-associated antigen and a target for anti-tumor immunity (85). Immunization with HSP-peptide complexes elicits potent T cell response against the chaperoned peptides and hence against the cells from which the HSPs are purified, as seen in studies with cancers (86). Since HSPs are potent immune-system stimuli, they could be used in vaccines as generic immune-system boosters, or adjuvants for treatment of cancer as well as infectious diseases.

CONCLUSION

The stress response represents a highly conserved defense program by which cells adapt to abrupt and adverse changes in their environment. Through the study of the structure/function of the stress proteins, especially those which function as molecular chaperones, the molecular basis for the acquisition and maintenance of protein conformation in the cell is now recognized. At the same time, there is increasing evidence that stress proteins play a crucial role in the protection of organs and tissues against injuries from surgery, ischemia/reperfusion, inflammation, or organ transplantation. Considering the potent cytoprotective action of stress proteins, nontoxic chaperone inducers may be of great therapeutic benefit as a new generation of drugs for the treatment of diseases.

ACKNOWLEDGMENTS

Our works, described in this review, were supported by a Grant-in-Aids for Scientific Research from the Japanese Ministry of Education, Science and Culture (Grant Nos.; 03454230, 05670481, 05268230, and 07670596) and by Eisai Co., Tokyo, Japan.

REFERENCES

1. Ritossa F : A new puffing pattern induced by temperature shock and DNP in Drosophila. Experientia 18 : 571-573, 1962
2. Linndquist S : The heat shock response. Annu Rev Biochem 55 : 1151-1191, 1986
3. In : Morimoto RI, Tissieres A, Georgopoulos C, eds : The biology of heat shock proteins and molecular chaperones. 2 nd edn, Cold Spring Harber Laboratory Press, Cold Spring Harber, New York, 1994, pp.1-593
4. Gething MJ, Sambrook J : Protein folding in the cell. Nature 355 : 33-45, 1992
5. Hendrick JP, Hartl FU : Molecular chaperone functions of the heat-shock proteins. Annu Rev Biochem 62 : 349-384, 1993
6. Becker J, Craig EA : Heat-shock proteins as molecular chaperones. Eur J Biochem 219 : 11-23, 1994
7. Hartl FU : Molecular chaperones in cellular protein folding. Nature 381 : 571-579, 1996
8. Blake MJ, Gershon D, Fargnoli J, Holbrook NJ : Discordant expression of heat shock protein mRNA in tissues of heat-stressed rats. J Biol Chem 265 : 15275-15279, 1990
9. Dienel GA, Kiessling M, Jacewicz M, Pulsinelli W : Synthesis of heat shock proteins in rat brain cortex after transient ischemia. J Cereb Blood Flow Metab 6 : 505-510, 1986
10. Dilmann WH, Mehta HB, Barrieux A, Guth BD, Neeley WE, Ross JJr : Ischemia of the dog heart induces the appearance of a cardiac mRNA coding for a protein with migration characteristics similar to heat-shock / stress protein 71. Circ Res 59 : 110-114, 1986
11. Anathan J, Goldberg AL, Voellmy R : Abnormal proteins serve as eukaryotic stress signals and trigger the activation of heat shock genes. Science 232 : 252-254, 1986
12. Kiang JG, Carr FE, Burns MR, McClain DE : HSP-72 synthesis is promoted by increase in $[Ca^{2+}]i$ or activation of G proteins but not pHi or cAMP. Am J Physiol 267 : C104-C114, 1994
13. Mivechi NF, Murai T, Hahn GM : Inhibitors of tyrosine and Ser / Thr phosphatases modulate the heat shock response. J Cell Biochem 54 : 186-197, 1994
14. Perisic O, Xiao H, Lis JT : Stable binding of Drosophila heat shock factor to head-tohead and tail-totail repeats of a conserved 5 bp recognition unit. Cell 59 : 797-806, 1989
15. Rabindran SK, Giorgi G, Clos J, Wu C : Molecular cloning and expression of a human heat shock factor, HSF1. Proc Natl Acad Sci USA 88 : 6906-6910, 1991
16. Sarge KD, Zimario V, Holm K, Wu C, Morimoto RI : Cloning and characterization of two mouse heat shock factors with distinct inducible and constitutive DNA-binding ability. Gene Dev 5 : 1902-1911, 1991
17. Schuetz TJ, Gallo GJ, Sheldon L, Tempst P, Kingston RE : Isolation of a cDNA for HSF2 : evidence for two heat shock factor genes in human. Proc Natl Acad Sci USA 88 : 6911-6915, 1991
18. Nakai A, Morimoto RI : Characterization of a novel chicken heat shock transcription factor, heat shock factor 3, suggest a new regulatory pathway. Mol Cell Biol 13 : 1983-1997, 1993
19. Nakai A, Tanabe M, Kawazoe Y, Inazawa J, Morimoto RI, Nagata K : HSF4, a new member of the human heat shock factor family which lacks properties of a transcriptional activator. Mol Cell Biol 17 : 469-481, 1997
20. Sarge KD, Murphy SP, Morimoto RI : Activation of heat shock gene transcription by heat shock factor 1 involves oligomerization, acquisition of DNA-binding activity, and nuclear localization and can occur in the absence of stress. Mol Cell Biol 13 : 1392-1407, 1993
21. Sistonen L, sarge KD, Morimoto RI : Human heat shock factors 1 and 2 are differentially activated and can synergistically induce hsp 70 gene transcription. Mol Cell Biol 14 : 2087-2099, 1994
22. Craig EA, Weissman JS, Horwich L : Heat shock proteis and molecular chaperons : mediators of protein conformation and turnover in the cell. Cell 78 : 365-372, 1994
23. Morimoto RI, Sarge KD, Abravaya K : Transcriptional

regulation of heat shock genes. J Biol Chem 267 : 21987-21990, 1992
24. Morimoto RI : Cell in stress : transcriptional activation of heat shock genes. Science 221 : 259 : 1409-1410, 1993
25. Cotto JJ, Kline M, Morimoto RI : Activation of heat shock factor 1 DNA binding precedes stress-induced serine phosphorylation : evidence for a multistep pathway of regulation. J Biol Chem 271 : 3355-3358, 1996
26. Jacquier-Sarlin MR, Polla BS : Dual regulation of heat-shock transcription factor (HSF) activation and DNA-binding activity by H_2O_2 : role of thioredoxin. Biochem J 318 : 187-193, 1996
27. Rokutan K, Hirakawa T, Teshima S, Honda S, Kishi K : Glutathione depletion impairs transcriptional activation of heat shock genes in primary cultures of guinea pig gastric mucosal cells. J Clin Invest 97 : 2242-2250, 1996
28. Welch WJ : Mammalian stress response : cell physiology, structure/function of stress proteins, and implications for medicine and disease. Physiol Rev 72 : 1063-1081, 1992
29. Minowada G, Welch WJ : Clinical implications of the stress response. J Clin Invest 95 : 3-12, 1995
30. Kampinga HH, Brunsting JF, Stage GJJ, Burgman PWJJ, Konings AWT : Thermal protein denaturation and protein aggregation in cells made thermotolerant by various chemicals : role of heat shock proteins. Exp Cell Res 219 : 536-546, 1995
31. Kabakov AE, Gabai VL : Heat shock-induced accumulation of 70-kDa stress protein (HSP70) can protect ATP-depleted tumor cells from necrosis. Exp Cell Res 217 : 15-21, 1995
32. Georgopoulos C, Welch WJ : Role of the major heat shock proteins as molecular chaperones. Annu Rev Cell Biol 9 : 601-634, 1993
33. Kampinga HH : Thermotolerance in mammalian cells. Protein denaturation and aggregation, and stress proteins. J Cell Sci 104 : 11-17, 1993
34. Jaattela M, Wissing D, Bauer PA, Li GC : Major heat shock protein hsp 70 protects tumor cells from tumor necrosis factor cytotoxicity. EMBO J 11 : 3507-3512, 1992
35. Ichijo H, Nishida E, Irie K, Dijke P, Saitoh M, Moriguchi T, Takagi M, Matsumoto K, Miyazono K, Gotoh Y : Induction of apotosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathway. Science 275 : 90-94, 1997
36. Verheij M, Bose R, Lin XH, Yao B, Jarvis WD, Grant S, Birrer MJ, Szabo E, Zon LI, Kyriakis JM, Haimovitz-Friedman A, Fuks Z, Kolesnick RN : Requirement for ceramide-initiated SAPK/JNK signalling in stress-induced apoptosis. Nature 380 : 75-79, 1996
37. Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME : Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 270 : 1326-1331, 1996
38. Gabai VL, Meriin AB, Mosser DD, Caron AW, Tis S, Shifrin VI, Sherman MY : Hsp70 prevent activation of stress kinases : a novel pathway of cellular thermotolerance. J Biol Chem 272 : 18033-18037, 1997
39. Polla BS, Mili N, Kantengwa S : Heat shock and oxidative injury in human cells. In : Marresca B, Lindquest S, eds. Heat shock. Springer-Verlag, Berlin, Heidelberg, New York, 1991, pp.279-290
40. Teshima S, Rokutan K, Takahashi M, Nikawa T, Kishi K : Induction of heat shock proteins and their possible roles in macrophages during activation by macrophage colony-stimulating factor. Biochem J 315 : 497-504, 1996
41. Jacquier-Sarlin MR, Fuller K, Dinh-Xauan AT, Richard MJ, Polla BS : Protective effects of hsp70 in inflammation. Experientia 50 : 1031-1038, 1994
42. Polla BS, Kantengwa S, Francois D, Salvioli S, Franceschi C, Marsac C, Cossarizza A : Mitochondria are selective targets for the protective effects of heat shock against oxidative injury. Proc Natl Acad Sci USA 93 : 6458-6463, 1996
43. Kroemer G, Zamzami N, Susin SA : Mitochondrial control of apoptosis : Immunol Today 18 : 44-51, 1997
44. Blake MJ, Udelsman R, Feulner GJ, Norton DD, Holbrook NJ : Stress-induced heat shock protein 70 expression in adrenal cortex : An adrenocorticotropic hormone-sensitive, age-dependent response. Proc Natl Acad Sci USA 88 : 9873-9877, 1991
45. Udelsman R, Blake MJ, Stagg CA, Li D, Putney DJ, Holbrook NJ : Molecular response to surgical stress : Specific and simultananeous heat shock protein induction in the adrenal cortex, aorta, and vena cava. Surgery 110 : 1125-1131, 1991
46. Udelsman R, Blake MJ, Stagg CA, Li D, Putney DJ, Holbrook NJ : Vascular heat shock protein expression in response to stress. J Clin Invest 91 : 465-473, 1993
47. Udelsman R, Blake MJ, Stagg CA, Holbrook NJ : Endocrine control of stress-induced heat shock protein 70 expression in vivo. Surgery 115 : 611-619, 1994
48. Chin JH, Okazaki M, Hu ZW, Miller JW, Hoffman BB : Activation of heat shock protein (hsp)70 and proto-oncogene expression by α1 adrenergic agonists in rat aorta with age. J Clin Invest 97 : 2316-2323, 1996
49. Xu Q, Li DG, Holbrook NJ, Udelsman R : Acute hypertension induces heat-shock protein 70 gene expression in rat aorta. Circulation 92 : 1223-1229, 1995
50. Xu Q, Fawcett TW, Udelsman R, Holbrook NJ : Activation of heat shock transcription factos 1 in rat aorta in response to high blood pressure. Hypertension 28 : 53-57, 1996
51. Ely DL : Organization of cardiovascular and neurohormonal responses to stress. Ann New York Acad Sci 594-608
52. Hauser GJ, Dayao EK, Wasserloos K, Pitt BR, Wong HR : HSP induction inhibits iNOS mRNA expression and attenuates hypotension in endotoxin-challenged rats. Am J Physiol 271 : H2529-H-2535, 1996
53. Nakamura K, Rokutan K, Marui N, Aoike A, Kawai

K : Induction of heat shock proteins and their implication in protection against ethanol-induced damage in cultured guinea pig gastric mucosal cells. Gastroenterology 101 : 161-166, 1991
54. Vass K, Welch WJ, Lindquest S : Localization of 70 kDa stress protein induction in gerbil brain after ischemia. Acta Neuropathol 77 : 413-424, 1988
55. Brown IR : Induction of heat shock (stress) genes in the mammalian brain by hyperthermia and other traumatic events : a current perspective. J Neurosci Res 27 : 247-255, 1990
56. Marcuccilli CJ, Miller RJ : CNS stress response : too hot to handle. Trends Neurosci 17 : 135-138, 1994
57. Chopp M, Chen H, Ho KL, Dereski MO, Brown E, Hetzel FW, Wech KMA : Transient hyperthermia protects against subsequent forebrain ischemic cell damage in the rat. Neurology 39 : 1389-1398, 1989
58. Kirino T, Tsujita Y, Tamura A : Induced tolelance to ischemia in gerbil hippocampal neurons J Cereb Bllod Flow Metab. 11 : 299-307, 1991
59. Kitagawa K, Matsumoto M, Kuwabara K, Tagaya M, Ohtsuki T, Hata R, Ueda H, Handa N, Kimura K, Kamada T : Ischemic tolerance phenomenon detected in various brain regions. Brain Res 561 : 203-307, 1991
60. Kitagawa K, Matsumoto M, Tagaya M, Hata R, Ueda H, Niinobe M, Handa N, Fukunaga R, Kimura K, Mikoshiba K, Kamada T : Hyperthermia-induced neuronal protection against ischemic injury in gerbils J Cereb Blood Flow Metab 11 : 449-452, 1991
61. Kuwabara K, Matsumoto M, Ikeda J, Hori J, Ogawa S, Maeda Y, Kitagawa K, Imuta N, Kinoshita T, Stern DM, Yanagi H, Kamada T : Purification and characterization of a novel stress protein, the 150-kDa oxygen-regulated protein (ORP150), from cultured rat astrocytes and its expression in ischemic mouse brain. J Biol Chem 271 : 5025-5032, 1996
62. Currie RW : Effects of ischemia and perfusion temperature on the synthesis of stress-induced (heat shock) proteins in isolated and perfused rat hearts. J Mol Cell Cardiol 19 : 795-808, 1987
63. Knowlton AA, Brecher P, Apstein CS : Rapid expression of heat shock protein in the rabbit heart after brief cardiac ischemia. J Clin Invest 87 : 139-147, 1991
64. Currie RW, White FP : Trauma-induced protein in rat tissues : a physiological role for á heat shock protein? Science 214 : 72-73, 1981
65. Delcayre C, Samuel JL, Marotte F, Best-Belpomme M, Mercadier JJ, Rappaport L : Synthesis of stress proteins in rat cardiac myocytes 2-4 days after imposition of hemodynamic overload. J Clin Invest 82 : 460-468, 1988
66. Izumo S, Nadal-Ginard B, Mahdavi V : Protooncogene induction and reprogramming of cardiac gene expression produced by pressure overload. Proc Natl Acad Sci USA 85 : 339-343, 1988
67. Locke M, Noble EG, Tanguay RM, Field MR : Activation of heat-shock transcription factor in rat heart after heat shock and exercise. Am J Physiol 268 : C 1387-C 1394, 1995
68. Donnelly TJ, Sievers RE, Vissern FLJ, Welch WJ, Wolf CL : Heat shock protein induction in rat hearts : a role for improved myocardial salvage after ischemia-reperfusion? Circulation 85 : 769-778, 1992
69. Yelon DM, Latchman DS : Stress protein and myocardial protection. J Mol Cell Cardiol 24 : 113-124, 1992
70. Karmazyn M, Mailer K, Currie RW : Acquisition and decay of heat-shock-enhanced postischemic ventricular recovery. Am J Physiol 259 : H424-H431, 1990
71. Currie RW, Karmazyn M, Kloc M, Mailer K : Heatshock response is associated with enhanced postischemic ventricular recovery. Circ Res 63 : 543-549, 1988
72. Currie RW, Tanguay RM, Kingma JJr : Heat-shock response and limitation of tissue necrosis during occlusion / reperfusion in rabbit hearts. Circulation 87 : 963-971, 1993
73. Marber MS, Latchman DS, Walker JM, Yellon DY : Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction. Circulation 88 : 1264-1272, 1993
74. Maber MS, Mestril R, Chi SH, Sayen MR, Yellon DM, Dillmann WH : Overexpression of the rat inducible 70-kD heat stress protein in a transgenic mouse increases the resistance of the heart to ischemic injury. J Clin Invest 95 : 1446-1456, 1995
75. Plumier JC, Ross BM, Currie RW, Angelidis CE, Kazlaris H, Kollias G, Pagoulatos GN : Transgenic mice expressing the human heat shock protein 70 have improved post-ischemic myocardial recovery. J Clin Invest 95 : 1854-1860, 1995
76. Suzuki K, Sawa Y, Kaneda Y, Ichikawa Y, Shirakura H, Matsuda H : In vivo gene transfection with heat shock protein 70 enhances myocardial tolerance to ischemia-reperfusion injury in rat. J Clin Invest 99 : 1645-1650, 1997
77. Hochstrasser M : Protein degradation or regulation : Ub the judge. Cell 84 : 813-815, 1996
78. Hayes SA, Dice JF : Roles of molecular chaperones in protein degradation. J Cell Biol 132 : 255-258, 1996
79. Vigh L, Literati PN, Horvath I, Torok Z, Balogh G, Glatz A, Kovcs E, Boros I, Ferdinandy P, Farkas B, Jaszlits L, Jednakovits A, Koranyi L, Maresca B : Bimoclomol : A nontoxic, hydroxylamine derivative with stress protein-inducing activity and cytoprotective effects. Nature Medicine 3 : 1150-1154, 1997
80. Hirakawa T, Rokutan K, Nikawa T, Kishi K. Geranylgeranylacetone induces heat shock proteins in cultured guinea pig gastric mucosal cells and rat gastric mucosa. Gastroenterology 111 : 345-357, 1996
81. Tuite MF, Lindquest SL : Maintenance and inheritance of yeast prions. Trends Genet 12 : 476-471, 1996
82. Born W, Happ MP, Dallas A, Reardon C, Kubo R, Shinnick T, Brennan P, O'brien R : Recognition of heat shock proteins and gamma / delta function. Immunol Today 11 : 1-4, 1990
83. Young DB : Heat-shock protein : immunity and autoimmunity. Curr Opin Immunol 4 : 396-400, 1992

84. DeNagel DC, Pierce SK : Heat shock proteins in immune responses. Crit Rev Immunol 13 : 71-81, 1993
85. Tamura Y, Tsuboi N, Sato N, Kikuchi K : 70 kDa heat shock cognate protein is a transformation-associated antigen and a possible target for the host s anti-tumor immunity. J Immunol 151 : 5516-5524, 1993
86. Tamura Y, Peng P, Liu K, Daou M, Srivastava PK : Immunotherapy of tumor with autologous tumor-derived heat shock protein preparations. Science 278 : 117-120, 1997