JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2007, 58, Supp 3, 43–62
www.jpp.krakow.pl

Review article

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HEAT SHOCK RESPONSE IN GASTROINTESTINAL TRACT

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Heat shock response is one of the defense mechanisms common to eukaryotic and prokaryotic cells. The highly conserved and ubiquitous heat shock proteins (HSPs) are essential for cell survival during stress. Stress tolerance, *i.e.*, adaptation of cells to stress conditions, is a characteristic feature of heat shock response. The lumen of the gastrointestinal tract is an external environment to the body. Epithelium of the digestive tract is exposed to various stress factors inducing the heat shock response, *e.g.*, bacteria and their toxins, food borne chemical compounds, drugs and diet deficiencies. Other factors like plant lecitins, glutamine or short fatty acids are mild stressors and can modulate the heat shock response in cells. All these factors are presumed to influence the normal microflora that is an integral part of the digestive tract. This review is focused on the induction/modulation of heat shock proteins expression in the epithelium of the gastrointestinal tract by various factors, on the protective role of HSPs and mechanisms leading to stress protection inside the gut. Heat shock response is one of the key mechanisms of maintenance of gastrointestinal tract homeostasis. It is involved in pathogenic bacteria adaptation to life in the digestive tract, especially in colony formation and in their role in infectious processes.

Key words: *heat shock proteins, stress tolerance, stress factors, gastrointestinal tract, microflora, pathogenic bacteria*

INTRODUCTION

Heat shock response (stress response), involving induction of heat shock proteins (HSPs) in cells under stress conditions, is a common phenomenon observed from bacteria to man. Heat shock proteins are strongly conserved and show high homology among all organisms. On the basis of their molecular size they have been divided into several families: hsp100, hsp90, hsp70, hsp60, hsp40 and small HSPs (1, 2). There are HSPs expressed in cells constitutively (*e.g.*, 70-

kDa heat shock cognate protein HSC70 also known as HSP73 or HSC73) as well as the stress-inducible forms (*e.g.*, HSP70 known as HSP72) (3-5). Besides elevated temperatures, there are many other stress factors inducing HSPs: environmental stresses (oxidative stress, toxic chemicals, heavy metals, UV radiation, nutritional deficiencies, changes in pH and osmolarity, electromagnetic fields, *etc.*), pathophysiological processes and diseases (fever, bacterial and viral infections, inflammation, ischemia and tumors), and physiological stress like exercise (2, 6-8).

There are two well defined families of heat shock proteins, molecular chaperones and proteases. Both of them enable cell survival in different ways. Proteases, *e.g.*, *Escherichia coli* Lon, Clp or HtrA, degrade damaged proteins. Chaperones maintain and control the native structure of proteins by protection and stabilization of stress-labile proteins, renaturation of denaturated proteins, and preventing protein aggregation. They are responsible for refolding of misfolded and aggregated proteins (6, 9). Proteins of all the HSPs families posses chaperone activities; however, the central and most important role is attributed to the hsp70 family (9, 10).

Much attention has been paid to the cytoprotective role of HSPs. Heat shock proteins adapt cells to stress conditions which leads to stress tolerance. Moreover, tolerance induced by one agent causes tolerance against agents of different origin and often more severe ones. This phenomenon called cross-tolerance is observed both in prokaryotic and eukaryotic cells (11, 12).

Two types of stress can be distinguished depending on its intensity: mild (*e.g.*, temperature of 39-42°C) and severe (*e.g.*, temperature of 43-45°C). Severe stress is destructive to the cell, causing cell cycle arrest and leading to apoptosis. Adaptation to stress conditions caused by mild stress is usually beneficial to the cell. Stress disturbs cell membrane fluidity that may result in activation of different signal transduction pathways, including the Ras signal pathways and mitogen-activated protein kinases (MAPK), that among others lead to induction of heat shock response (13).

Cytoprotection induced by heat shock proteins involves inhibition of apoptosis. The apoptotic pathway can be blocked at several levels and prevent the activation of specific proteases known as caspases, the cell executors of apoptosis (14, 15). There are many examples of cytoprotection caused by HSPs, particularly those of the hsp70 family: protection of the heart against subsequent damage after ischemia and reperfusion (cardioprotection) (16); increased survival of neurons in ischemic stroke of the brain, protection of neurons from amyloid toxicity in Alzheimer's disease, prevention of aggregation of misfolded proteins in neurons in Huntington's disease (17).

Induction of HSPs by specific drugs as a possible treatment for various diseases is discussed (12, 17, 18).

Stress tolerance seems to be also the result of modulation of immune response by heat shock proteins. HSPs participate in antigen presentation and intercellular signaling. In this way they mobilize the immune system during

bacterial or viral infection as well as participate in induction of tumor-specific immunity (14). On the other hand, HSPs show antiinflammatory properties by inhibiting of cytokinin production (IL6, IL8) and prevent the immune response destruction of the cells (14, 19).

An example of HSP-induced protection of bacterial cell is the significant increase in survival of UV-irradiated bacteria due to expression of heat shock proteins. It probably reflects enhanced processes of DNA repair (20-22).

Molecular chaperones perform several housekeeping functions not only under stress but also under physiological conditions. They are responsible for protein folding, assembly, maturation and stabilization of newly synthesized peptides; translocation and transport of proteins; control of the activity of regulatory proteins like transcription factors, replication proteins or kinases. They play an important role in cell cycle, cell growth, development and differentiation (2, 6).

In all organisms expression of heat shock proteins is regulated at the transcriptional level. In bacteria promoters of heat shock response regulon are recognized by specific subunits of RNA polymerase; in *Escherichia coli* it is 32-kDa sigma factor, σ^{32}, encoded by *rpoH* gene. In vertebrates stress causes activation of specific transcription factors, HSFs, that bind to the promoter element (HSE) of heat shock response genes. Among transcription factors, HSF1 is the main one. In lower eucaryotes there is one HSF factor, equivalent to HSF1. Under normal conditions HSPs cause inhibition of the heat shock response. In eukaryotic cells they bind to HSF1 making it an inactive monomer. In bacterial cells they bind to σ^{32} subunit preventing interaction with RNA polymerase, and to the σ^{32} mRNA blocking translation. Stress-damaged proteins recruit HSPs. In eukaryotic cells this leads to derepression of HSF1, its translocation to the nucleus, hyperfosforylation and formation of an active trimer. In bacterial cells there is an increase in σ^{32} level, due mainly to σ^{32} mRNA translation rather than to *rpo*H gene transcription. Both in prokaryotic and eukaryotic cells heat response is suppressed in a negative feed-back regulatory system. More extreme stress conditions cause the expression of sigma E (σ^{E}) factor. The σ^{E} regulon members are the *rpoH* or *htrA* genes, the latter encoding the HtrA protease (6, 23).

HEAT SHOCK RESPONSE IN THE GIT ORGANS

Stress factors and the protective role of heat shock response in GIT

The lumen of the gastrointestinal tract (GIT) is an external environment to the body. Organs of the digestive tract are exposed to different stresses like pathogens and their toxins, chemical compounds like food pollutants, food coloring dyes, preservatives, drugs, improper diet, etc. The epithelium of digestive tract is the largest area through which the organism contacts directly with the external environment and becomes a barrier protecting the body against harmful factors. It is particularly important in the small intestine where absorption takes place and

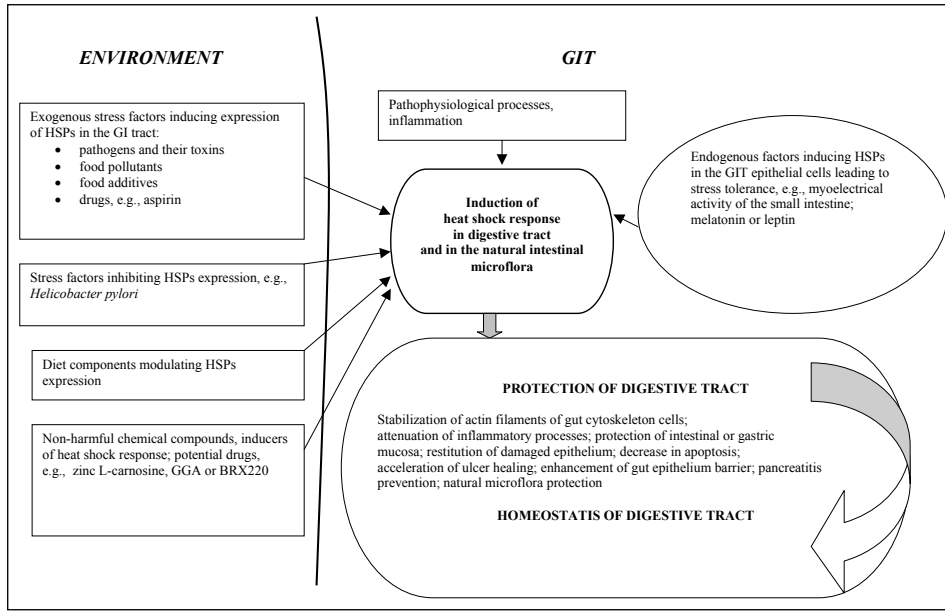

Fig. 1. Induction and modulation of HSPs expression in GIT epithelial cells and the role of HSPs in maintenance of digestive tract homeostasis.

the area of epithelium is significantly extended due to the villi structure. Moreover, GIT is a complex microbial ecosystem. Bacteria living there become the normal gut microflora and are an integral part of the digestive tract essential for its proper functioning. Normal flora (also called autochthonous microbiota) is a population of microbes that normally reside in the gut and live in harmony with the host (24). It could be expected that stressors to digestive organs also influence the normal flora colonizing the digestive tract. Hence, there must exist defense mechanisms, both in epithelial and bacterial cells, that ensure homeostasis of the gastrointestinal tract. Heat shock response is regarded as one of them (*Fig. 1*).

Pathogenic bacteria

Pathogens and their toxins in the gastrointestinal tract are unquestioned stress factors. Induction of HSPs by pathogenic bacteria and their toxins has been determined in Caco-2 cells. The enterocyte-like Caco-2 cell line of human colon carcinoma is a useful *in vitro* model of intestinal epithelium due to its structural and functional similarities to enterocytes. It has been shown that *Salmonella enteriditis* 857, but not its endotoxin, induces HSP70 and HSP90 in Caco-2 cells (25). Other authors have reported induction of HSP72 in Caco-2 cells by *E. coli* C25 and its endotoxin C25 (26). Similar results (induction of HSP70 and HSP90) have been obtained for B4 endotoxin from pathogenic *E. coli* 001 strain (25). It

is known that pathogenic bacteria destroy actin filaments of the gut cytoskeleton cells. Stabilization of the Caco-2 cytoskeleton and decrease of pathogenic adhesion to the Caco-2 cells seems to be the role of heat shock proteins.

A serious stressor for gastric mucosa is the *Helicobacter pylori* infection. To cope with the acidic conditions in the stomach, *H. pylori* locally increases the pH in its microsurroundings by its urease activity resulting in production of ammonia (NH_4OH). The bacteria activate neutrophils that produce reactive oxygen species, mainly superoxide (O_2^-) and hydrogen peroxide (H_2O_2). Oxidation of chloride (Cl^-) by H_2O_2 yields hypochlorous acid (HOCl). The HOCl reacts with bacteria produced ammonia generating highly reactive and cytotoxic monochloramine (NH_2Cl) that leads to mucosal injury and DNA fragmentation. The *H. pylori* infection causes an increased apoptosis rate and loss of cells from the epithelium surface leading to mucosal damage, chronic gastritis, atrophy and, in some cases, cancer. These processes are mediated by monochloramine. The influence of monochloramine on cell injury was investigated in rat gastric mucosal cells (RGM-1) overproducing HSP70 from the plasmid bearing a full length human HSP70 gene. The expression of HSP70 in these cells was at 350% of the level in the control cells. It has been shown that overexpression of HSP70 significantly protects gastric cells against NH_2Cl-induced injury and decreases the rate of necrosis and apoptosis in comparision to the control cells. The results indicate that HSP70 plays an important role in protection of gastric epithelial cells against monochloramine-induced injury, and overproduction of HSP70 constitutes a therapy for *H. pylori*-associated gastric mucosal injury (27). On the other hand, during colonization of the host digestive tract *H. pylori* can abolish the expression of the host heat shock proteins. It was shown that *H. pylori* is able to attenuate the heat shock response in KATO III cells. KATO III is a gastric carcinoma cell line used as a model of human gastric epithelium. The bacteria produce a yet unidentified X factor promoting the formation of inactive complexes of HSF1 and the phosphorylated form of STATs (signal transducers and activators of transcription). As a result, transcription of heat shock genes in KATO III is abolished. In KATO III *H. pylori* also induces apoptosis, probably as a result of inhibition of heat shock proteins, including HSP70, that prevent apoptosis (28). The authors hypothesize that increased apoptosis is a mechanism that eliminates host cells damaged or altered by *H. pylori* infection. Other studies (29) on the human gastric adenocarcinoma cell line MKN7 revealed involvement of *H. pylori* CagA protein in attenuation of the HSP70 mRNA expression. The CagA is a bacterial cytotoxin important but not essential for *H. pylori* pathogenicity. The *cagA* and encoding another cytotoxin, *vacA* genes are members of *cag* PAI pathogenicity island. The island is a 40 kb locus containing up to 27-31 genes encoding so called type IV secretion system (TFSS) being responsible for translocation of bacterial proteins into eukaryotic cells (29).

A sequence of stress factors seems to play a key role in the development of stress tolerance. Cytoprotection is observed when severe stress is followed by mild

heat stress whereas a reverse sequence of stress action leads to apoptosis. The combined effect of two stresses, mild (elevated temperature) and severe (bacterial lipopolysaccharides, LPS) was examined in the rat intestinal epithelial cell-6 (IEC-6) line. A significantly increased level of apoptosis and rate of cell damage were observed when the cells were first treated with LPS and then with elevated temperature. The reverse sequence of stressors' application (high temperature as the first stress factor) caused development of stress tolerance to LPS. In this case, mild stress, when acting first, plays the role of a preconditioning factor. The described observation reflects a phenomenon called the heat shock paradox (30).

Drugs and chemical compounds

Many data show the protective role of HSPs against damage of GIT epithelium and GIT organs caused by various drugs and chemicals.

In pancreas of rats and mice during acute pancreatitis the decreased level of HSPs was observed. Acute pancreatitis is caused by intrapancreatic activation of digestive zymogens. Pancreatitis-induced compounds like cerulein or cholecystokinin (CCK) are known to cause significant decrease of HSPs in rat pancreas *in vivo* (31, 32) and *in vitro* in the rat pancreatitic accinar cell line, AR42J cells (33, 34). Preinduction of HSPs in pancreatic tissue diminishes effects of pancreatitis by increasing antioxidant properties, stabilization of acin filaments, increasing the resistance of the cells to apoptosis, decreasing pro-inflammatory cytokine levels and preventing intracellular trypsinogen activation.

Much attention was paid to the protective role of HSP60, HSP70 and HSP27 in pancreas. It was shown that specific preinduction of HSP60 by water immersion stress in rats prevented development of cerulein-induced pancreatitis. Moreover, when water immersion stress was applied after cerulein injection, the cerulein-induced pancreatitis was exacerbated (31, 35) so the results resemble the heat shock paradox observed by Xu and co-workers (30) in the rat epithelial cells. Protective role of HSP60 was also demonstrated in cerulein-treated AR42J cells (33, 34).

It was shown that non-toxic heat shock protein inducer BRX-220 had a protective effect against CCK-induced acute pancreatitis in rats presumably due to expression of HSPs, HSP60 and HSP72. Application of HSP-inducing drugs are considered as a treatment of acute pancreatitis (32).

Induction of HSP70 by hyperthermia prevented cerulein-induced pancreatitis in rats. Administration of a specific anti-HSP70 oligonucleotide to those rats reduced the protective effect of hyperthermia due to inhibition of HSP70 expression. Rats not subjected to the thermal stress revealed exacerbation of the pancreatitis indicating to the protective role of HSP70 in pancreas (36). From the other side, Otaka and co-workers (31) observed no protective effect of preinduction of HSP72 by hyperthermia on cerulein-induced pancreatitis in rats.

Protection against cerulein-induced acute pancreatitis was also observed in transgenic mice overexpressing human HSP27 in pancreas. HSP27 plays an

important role in stabilization of the actin cytoskeleton due to regulation of actin polymerization (37).

The role of heat shock proteins in protection of the colonic mucosa and small intestine against acetic acid-induced mucosal damage in rats was confirmed under conditions of hyperthermia. Elevated temperature significantly decreased acetic acid-induced colitis. This protective effect was mainly attributed to the synthesis of HSP72 and HSP90 whereas induction of HSP60 did not clearly correlate with colonic mucosa protection (38). Moreover, preinduction of HSP60 by water immersion stress did not show any cytoprotective effects on acetic acid-induced small intestinal lesions in rats (39). The results suggest different functions of HSPs in different organs of GIT.

Experiments on cultured guinea pig gastric mucosal cells have shown that sublethal heat stress treatment dramatically reduced ethanol-induced damage. Inhibition of protein synthesis by cyclohexamide eliminated this protective effect (40). The same protection of cultured guinea pig gastric mucosal cells against ethanol-induced damage was observed after administration of geranylgeranylacetone (GGA). The *in vivo* and *in vitro* studies have shown that GGA is an antiulcer drug that induces resistance of gastric mucosal cells to irritants *in vivo* and *in vitro* due to activation of HSF1 and HSP70 expression (41).

It is known that long-term repeated administration of non-steroidal antiinflammatory drugs like aspirin causes increased resistance of gastric mucosa to the damage induced by those drugs. It was shown that administration to rats of a low dose of aspirin (100 mg/kg) caused expression of HSP72 in gastric mucosa whereas no HSP60 and HSP90 were induced. The longer the time of aspirin administration, the higher was the level of HSP72 expression. Moreover, the induction significantly correlated with protection of cells against damage caused by high dose of aspirin (250 mg/kg). This indicates that HSP70 plays an important role in gastric adaptation to aspirin. Long term administration of a low dose of aspirin was a preconditioning factor providing stress tolerance against severe stress caused by a higher dose of the drug (42). However, in humans adaptation of gastric mucosa to aspirin can be disturbed by *H. pylori* infection. Analysis of gastric mucosal biopsies from *H. pylori* negative and positive patients revealed reduced expression of HSP70 during *H. pylori* infection. This reduced expression of HSP70 is the reason of disturbances in adaptation of gastric mucosa to aspirin. Elimination of the pathogen has restored both the normal HSP70 protein expression and the process of adaptation to aspirin (43).

Non-toxic compounds inducing heat shock response may be important for the development of new therapeutic strategies for colonic inflammatory diseases (44). Good candidates are *e.g.* zinc L-carnosine and mentioned above GGA or BRX220. Cytoprotective effects of zinc compounds on gastric mucosa are known, and zinc L-carnosine (polaprezinc) is an antiulcer drug preventing gastric lesions. It has been shown that both zinc L-carnosine and zinc sulphate cause induction of HSP72 in in cultured rat gastric mucosal cells (RGM-1) *in vitro* and

in rat gastric mucosa *in vivo*. However, the zinc L-carnosine-induced expression of HSP72 *in vivo* was more effective than that of zinc sulphate. The results indicate that gastric mucosa protection against different irritants is the result of heat shock proteins induction (45).

Inflammation and epithelial damage

During inflammation the enterocytes of intestinal epithelium secrete interleukins: IL6, IL8, IL-1β or tumour necrosis factor α (TNFα). The control of this secretion is important in prevention of chronic inflammation and epithelial cell damage. HSPs play a key role in protection against inflammation by inhibition of proinflammatory cytokine production. Experiments with Caco-2 cells have shown that pretreatment of the cells with elevated temperature (42°C) to express heat shock proteins significantly decreases interleukin 8 production after infection with *S. enteritidis 857* (19). The role of heat shock proteins during inflammation is complex and involves selective interference in pathways mediating cytokine production and growth response (MAPK or nuclear factor kappa B NF-κB pathways). Besides inhibition of proinflammatory cytokine production, HSPs promote cellular proliferation and, as a result, restitution of the damaged epithelium (46).

On the contrary interleukin 11 (IL11) is known to play a protective role in the intestinal mucosa during inflammation or intestinal injury due to modulation of immune response, reduction of proinflammatory cytokines production and antiapoptotic activity. Using the rat intestinal epithelial IEC-18 cells and young adult mouse colon cells (YAMC) it was demonstrated that IL11 selectively increases HSP25 expression making the cells more resistant to oxidative stress caused by monochloramine. Oxidative stress is a characteristic feature of inflammation and intestinal injury. Introduction of HSP25 anti-sense plasmid to the IEC-18 cells reduced the protective effect of interleukin 11. Moreover, the selective induction of HSP25 expression by IL11 seems to be intestinal epithelial-specific cytoprotective mechanism since it was observed neither in non-intestinal epithelial nor in non-epithelial control cell lines (47).

It is thought that healing process of gastric ulcers is similar to that of wound healing. In acetic acid induced gastric ulcers in rats the level of HSC70, HSP70 and HSP47 expression was investigated during healing process. Gradual, connected with ulcer healing increased expression of HSP70 in the ulcer margin was observed, whereas the level of HSC70 was at the same level in normal mucosa and ulcer at every step of ulcer development and healing. Moreover, enhancement of HSP70 expression was shown after treatment with omeprazole, a drug accelerating ulcer healing. High level of HSP70 expression detected in the proliferating cells of regenerated mucosa indicates its role in mucosal regeneration during process of ulcer healing (48).

The influence of overexpression of HSP70 from plasmid DNA on artificial wound restoration in RGM-1 cells has also been studied. The results have shown that wound restoration was significantly enhanced in HSP70-overexpressing cells in comparison to the cells without the plasmid. Moreover, mechanical stretch inhibited wound restoration and the level of HSP70 in the control cells, but had no effect on the HSP70-overexpressing cells. These results confirm participation of HSP70 in gastric wound healing, for example, in gastric ulcers. The movement of food stored in stomach is a mechanical stretch that influences gastric mucosal cells (49).

It was also demonstrated that heat shock preconditioning prevented apoptosis in superficially injured isolated guinea pig gastric mucosa. The observed decrease in apoptosis was mainly due to inhibition of caspase-3 activity by HSP60 and HSP70 (50).

Studies on inflammation processes and intestine epithelium damage may be significant for the search for therapeutic treatment of bowel inflammation diseases, including, the Crohn's disease.

HSPs also participate in regeneration of whole organs. In mice after hepatectomy increased level of HSP27, HSP60, HSP70 and HSP90 in the early phase of liver regeneration has been observed. Moreover, intraperitoneal injection of quercetin, a HSPs inhibitor, led to a delay in this process. Obtained results indicate that HSPs play a significant role in the liver regeneration. Protective role of HSPs presumably involves inhibition of apoptosis, chaperone properties, activation of Kupffler cells in production of cytokines (IL6, TNF-α), stimulation hepatocyte proliferation. It has to bee stressed that liver regeneration is a very important physiological process in a human body (51).

Endogenous mechanisms of preconditioning causing adaptation to stress in the digestive tract

In GIT there are many different stress factors inducing heat shock response in epithelial cells. Induction of heat shock proteins by any inducer, overproduction of HSPs or, most often, preconditioning with mild stress lead to stress tolerance. Preconditioning seems to be beneficial and even necessary for cell survival. Cells stressed by a sublethal insult inducing HSPs are rendered more resistant to subsequent extreme stress. This suggests that natural endogenous mechanisms of preconditioning causing adaptation to stress in GIT must exist.

Some endogenously produced substances like melatonin or leptin are known to play a significant role in protection of the digestive tract organs. Melatonin, a main product of the pineal gland, is also synthesized in the GIT by enterochromaffine cells. Leptin, a product of OB gene synthesized mainly by adipopcytes, is a regulatory protein involved in the control of body weight, food intake and energy expenditure. Studies on the rat pancreatic acinar cell line AR42J indicate that the protective effect of both melatonin and leptin is probably achieved, among others, by stimulation of HSPs production. HSP60 gene

expression was significantly increased in AR42J cells after incubation with melatonin or leptin. These substances protect the cells against cerulein-induced damage suggesting their protective role in the pancreas (33, 34).

Several studies have shown that different organs of the GIT express HSPs on different levels in non-stressful conditions. The level of constitutive heat shock proteins HSC73 and HSP84 and stress-inducible HSP25 and HSP71 in many organs of young and adult mice was examined under non-stress conditions. The level of constitutive forms of HSC73 and HSP84 was the same in all tissues except the skin, whereas expression of HSP25 and HSP71 showed a tissue-specific pattern and was the highest in organs such as stomach, intestine, colon and bladder, potentially most exposed to toxic environmental substances or toxic metabolites. No differences were observed between young and adult animals (52).

Katsuki and co-workers (53) have found that feeding induces a heat shock response regulated by the HSF1 transcription factor in mice liver and small intestine, but not in colon, stomach, brain, muscle and lung. Expression of HSP70 in the intestine was higher than in the liver. Since the liver plays a key role in detoxification of chemicals present in food, the authors studied the stress response in this organ more precisely, and found, besides HSP70, significant induction of HSP90 and HSP27. A novel mechanism protecting the digestive organs against xenobiotic chemicals in food causing oxidative stress has been suggested. Since digested elements are adsorbed mainly in the intestine and are transported to liver by blood through the portal vein, these two organs seem to be most exposed to xenobiotics. This may be the reason for induction of stress response only in the liver and small intestine.

Myoelectrical activity of GIT

The induction of HSP60 by water-immersion stress or thyrotropin-releasing hormone (THR) administration has not led to any pathologic changes in rat small intestine. Similarly no changes were observed in the colonic mucosa and pancreas in rats. THR belongs to the group of endogenous substances released during stress, such as catecholamine, β-endorphin, 5-hydroxytryptamine or corticotrophin-releasing factor. It was presumed that the expression of HSP60, although not of HSP72 or HSP90, was the result of increments of intestinal motility during stress. The authors hypothesized that intestinal hypermotility causes mild ischemia that could induce HSP60 (31, 39).

Results described above are consistent with our findings that myoelectrical activity of the small intestine induces a heat shock response in Caco-2 cells and *E. coli* (54). Gastrointestinal smooth muscles like any other muscular tissues produce extremely low electric currents and electric fields (EF) due to changes in their membrane potentials that are closely related to gut contractions and motility. The interstitial Cajal cells placed between the smooth muscle layers of the small intestine generate and conduct slow waves to smooth muscle cells (55). The basic

pattern describing the myoelectrical activity of the small intestine is a myoelectrical migrating complex (MMC) consisting of three phases: quiescence phase I with no spiking activity, and phases II and III with spiking activity and different intensity of generating electric fields (56).

Epithelial cells of GIT and also bacteria living therein, particularly those adhering to the epithelium, are presumably exposed to the MMC-related EF (54, 57). It has been shown that MMC-related EF causes expression of HSP70 protein in Caco-2 cells and σ^{32} factor in *E. coli* cells (54). Moreover, this intestinal EF induces and protects the bacteria against UV irradiation making them more resistant to UV light. This protective effect was not observed in the *E. coli dnaK dnaJ* mutant that is deprived of heat shock proteins, DnaK (hsp70 family member) and DnaJ (hsp40 family member), pointing to the involvement of heat shock response in MMC-related EF protection (54, 58). We suppose that alterations in electric field connected with the three phases of different electrical activity and changes in electric properties of the cell membranes constitute the heat shock response inducing factor. Experiments on calves showed that the intestinal myoelectrical activity is synchronized with motility and digesta flow. In the quiescence phase I there is no digesta flow whereas its maximum is observed before phase III (59, 60). It is also known that bacterial toxins and different harmful chemicals enhance gut motility, particularly the duration and migration of phase III (61, 62). We presume that the heat shock response induced by feeding in the small intestine and the liver in mice observed by Katsuki *et al.* (53) was also caused by gut motility connected with digesta flow.

According to our hypothesis the role of myoelectrical activity of the small intestine is the protection of intestinal epithelial cells as well as natural flora against toxins and harmful substances in food by induction of heat shock proteins. We suggest that it is a sort of mild stress producing cross-tolerance to subsequent stronger stress agents of different type.

Effect of diet components on induction of heat shock response in digestive tract

Natural components of diet can modulate heat shock response in cells. It is well known that glutamine, conditionally essential amino acid, increases the heat shock response in many organs under normal as well as under stress conditions. In experiments with the intestinal cell line IEC-18 it has been shown that glutamine protects intestinal epithelial cells against oxidative damage (63). Cell survival of intestinal epithelial cell culture IEC-6 has been enhanced under non-stress conditions due to glutamine supplementation. Moreover, glutamine protected these cells against lethal heat shock and reduced the number of dead cells after extreme thermal stress treatment. Glutamine is also important in intestinal cell proliferation. These results indicate that glutamine prevents intestinal mucosal atrophy (64). Among HSPs especially high expression of two proteins, HSP25 and HSP72, has been observed under the influence of glutamine. Glutamine-supplemented diet is

thought to be beneficial to man. Intravenous glutamine is a treatment for patients suffering from critical illness and injury like, for example, sepsis (65). It was shown that glutamine enhances induction of HSP25 in the rat intestinal epithelial cells (IEC-18) after heat treatment. Such amino acid-directed increase of HSP expression was not observed when glutamine was exchanged for other amino-acids like arginine, histidine, proline, leucine, asparagine or tyrosine which confirms that glutamine supplementation optimize cellular response to stress (66). Recently, much attention has been focused on the role of glutamine in attenuation of inflammatory processes and enhanced protection of the gut epithelium barrier by decreasing gut permeability due to induction of heat shock response (67).

Other factors enhancing heat shock response are short chain fatty acids (SCFAs), *e.g.*, butyrate. SCFAs are components of colonic fluid derived from anaerobic metabolism of luminal carbohydrates by bacterial flora. Butyrate is the most abundant short chain fatty acid in the colon lumen. Its physiological concentrations in man range from 5 to 24 mM and depend on the fiber contents in diet. Significant enhancement of HSP70 expression in Caco-2 cells cultivated at increasing physiological butyrate concentrations (0.05-20mM) has been observed, and the response is higher in crypt-like (undifferentiated) than in villous-like (differentiated) cells (19). Butyrate-induced, time- and concentration-dependent selective increase of HSP25, but not HSP72, expression, protecting the cells against oxidative damage, has been also reported in rat ICE18 cells (68). Butyrate deficiency has been found to result in inflammatory bowel diseases and pathogenesis. On the other hand, it is known that butyrate can inhibit or promote, depending on its concentration, intestinal inflammation via modulation of IL8 production. In Caco-2 cells low butyrate concentrations suppress IL8 production, whereas higher concentrations increase it. It should be stressed that in patients with active Crohn's disease high butyrate concentrations and high levels of IL8 are found in intestinal mucosa (19).

Plant lecitins are another heat shock response modifying factor in GIT that decreases the expression of heat shock proteins. Lecitins are known to cause structural lesions in digestive tract epithelial cells leading to disturbances in the integrity of the epithelial layer. It was reported that lecitins, PHA from kidney bean (*Phaseolus vulgaris*) and WGA from wheat germ (*Triticum aestivum*), decreased the level of HSP70 and HSP90 in heat induced Caco-2 cells. Moreover, constitutive level of HSPs in intestinal cells of rats fed with diet containing PHA and WGA lecitins was lower than in the control group. Lecitins binding to membrane receptors probably cause disturbances in signal transduction resulting, among other factors, in inhibition of heat shock proteins expression (69).

Inadequate diet, deficient in some components, is a stress factor to the whole organism including epithelial cells. The influence of organic selenium as diet supplement on HSP70 expression in ileum and liver has been tested in broiler chickens. Elevated temperature or entheropathogenic *E. coli* (EPEC) has been used as a stress factor. Stress conditions caused increased expression of HSP70 in chickens fed with and also without organic selenium. However, the level of

HSP70 was significantly higher in the latter case and the health of these chickens was much worse (inhibition of growth, increased rate of mortality). Organic selenium plays an important role in protection against reactive oxygen species by regulation and control of glutathione peroxidase GPx activity and glutathione level in cells. Selenium deficiency increases oxidation stress in cells. Reduced growth rate in chronically stressed animals (*e.g.*, by inadequate diet) is in part is the result of a lower rate of structural protein synthesis, which is the metabolic cost of increased synthesis of HSP proteins (70).

The weaning, involving dietary, environmental and psychological changes, constitutes a stress for young animals. It reflects histological and biochemical changes in the small intestine, like partial villous atrophy, crypt hyperplasia and decreased activity of digestive enzymes, that lead to reduced feed intake, disorders in digestion and absorption, post-weaning diarrhea or growth check. Expression of four HSPs, HSP27, HSC70, HSP70 and HSP90, was examined along the gastrointestinal tract of suckling piglets aged 21 and 28 days, both before and after weaning. It was shown that in suckling piglets all the examined HSPs were expressed constantly in every region of the gastrointestinal tract. Weaning caused changes in HSP27, HSP70 and HSP90 expression, but not in HSC70 according to spatial-temporal pattern. The role of heat shock proteins seems to be protection of young animals' digestive tract against stress due to weaning and changes in diet (71).

There is no doubt that the role of stress response in gastrointestinal tract homeostasis is very important since both excess and lack of HSP expression lead to serious consequences. Much attention is being paid to the development of new therapeutic strategies for colonic inflammatory diseases and digestive tract epithelium injury, including different drugs (potent chaperone inducers and other factors modulating the heat shock response) or chaperone-inducing gene therapy. HSPs not only protect cells by acting as chaperones but also downregulate proinflamatory cytokine production, decreasing inflammation processes. The resulting heat shock response induced cytoprotection restores the function of intestinal epithelium. However, it should be stressed that heat shock response is only a part of the complex and complicated network of mechanisms responsible for digestive tract homeostasis. There are many mechanisms maintaining GIT homeostasis like the intestinal immune system, including cytokines, chemokines and anibodies; barrier function of the intestinal mucosa; pancreatic and gastric blood flow (72).

ROLE OF HEAT SHOCK RESPONSE IN ADAPTATION OF BACTERIA TO LIFE IN THE DIGESTIVE TRACT

Induction of heat shock response in bacteria has been studied most intensively in *E. coli* growing exponentially under aerobic conditions. However, the natural

environment of *E. coli* is an anaerobic digestive tract where most bacteria are in the stationary phase of growth. It was shown that in bacteria growing under anaerobic conditions the heat shock response is stronger and heat shock proteins persist in a cell for a longer time. Moreover, it seems that σ^{32}- and σ^{S}- independent mechanism plays an important role in bacterial cell protection against heat shock. Mutations in the genes encoding these factors (*rpoH* and *rpoS*, respectively) were insensitive to 47°C in the exponential growth phase under anaerobic conditions. In the stationary growth phase σ^{70} subunit of RNA polymerase is exchanged by σ^{S}, which ensures resistance to environmental stress characteristic for this stage of growth. Admittedly, in the stationary phase the expression of heat shock proteins after thermal stress is delayed, but instead the proteins last longer (73).

Lactic acid bacteria (LAB) are a heterogeneous group involving probiotic species, namely bacteria that confer health benefits on the host, such as immunostimulation, pathogen exclusion and maintenance of normal microflora. LAB are essential components of milk and other fermented food products and dietary supplements. They are able to colonize host GIT. Stress factors cause expression of heat shock proteins and stress tolerance in LAB and other Gram-positive bacteria; however, global regulator of the heat shock response or stress-specific sigma factors have not been identified yet. Probably different stress regulated networks are involved. Highly conserved chaperones, DnaK, DnaJ, GrpE, GroES, GroEL, and proteases, Clp, HtrA and FtsH, have been detected in LAB (74). In GIT the most common stress factors influencing LAB are heat, osmotic stress, low pH and bile salts. It has been shown that elevated heat shock proteins and/or chaperone production improve performance of probiotic LAB. Studies have been done on *Lactobacillus acidophilus* (75), *Lactococcus lactis* and *Lactobacillus paracasei* (76), *Lactobacillus rhamnosus* (77) and *Lactobacillus delbrueckii subsp. bulgaricus* (78). The results have shown that stationary phase LAB cultures are more resistant to stress conditions (75).

Several data indicate that heat shock response is one of the mechanisms enabling pathogenic bacteria to colonize digestive tract during infection. Interesting studies have involved *Campylobacter jejuni*, a Gram-negative human pathogen whose natural environment is bird digestive tract with temperature of about 42°C. The C. *jejuni dnaJ* mutant is not able to colonize chicken GIT (79). Whole genome DNA microaray showed that growth at 42°C leads to induction of *C. jejuni* heat shock genes: *lon, clpB, hslU, groEL, groES, grpE, dnaK* and *dnaJ* (80). A source of infection in humans is fecal contaminated poultry meal. During transmission from poultry GIT to human gut, *C. jejuni* is subjected to stress conditions, *e.g.*, oxygen stress. It has been shown that HtrA protease, responsible for degradation of denaturated or misfolded proteins, is essential for termal or oxygen stress tolerance as well as for interaction with human intestinal epithelial cells. In the *htrA* mutants adherence to and invasion of human epithelial cell line INT407 have been significantly reduced (81). Genome-wide expression profile of *C. jejuni* during host colonization and pathogenic development in the rabbit ileal

loop model (a mammalian model of gastroenteritis) also revealed that heat shock proteins are required for bacterial colonization of intestinal tract (82).

In *S. enterica*, an enteric pathogen causing gastroenteritis, heat shock response genes are under control of sigma factor H (σ^H), a heat shock specific σ subunit of RNA polymerase. Another sigma factor, σ^E, is essential for survival under extreme stress conditions and in virulence. It has been shown that *clpX, clpP, lon* and *rpoE* mutants of *S. enterica* are not able to cause systemic infection of mice and survive in peritoneal macrophages *in vitro*. ClpXP and Lon proteins, the main bacterial proteases, are necessary for the survival and growth of bacteria within macrophages probably due to degradation of proteins damaged by the host defense system during infection and regulation of expression of invasion proteins (83, 84). The key role of DnaK/DnaJ chaperone machinery in systemic infection of mice by *S. enterica* was also demonstrated. *S. enterica dnaK* mutant did not survive or proliferate in mouse macrophages, was unable to invade epithelial cells and showed disturbances in secretion of virulence factors (85). Similarly to *C. jejuni*, when *Salmonella* colonize bird GIT, heat shock response also plays a significant role adapting the bacteria to the higher body temperature of avian host.

Induction of heat shock response and tolerance of stress-adapted bacteria to more extreme treatments of various stress factors are also observed in other food borne pathogens that have to cope with stress conditions in the host digestive tract. The most often studied stress factors are elevated temperature, osmotic stress, bile salts and low pH. The examples of such pathogens are *Enterococcus faecalis* (86) or *Listeria monocytogenes*. It was reported that *Listeria monocytogenes* mutated in sigma B (sigma factor recognizing promoters of heat shock response genes) or HtrA protease genes show attenuated virulence (87, 88).

Non-pathogenic as well as pathogenic bacteria developed many mechanisms for coping with stress factors in digestive tract. Heat shock response is one of them. All the protective mechanisms have been well described for *S. enterica* (89) and lactic acid bacteria (74).

Studies on mechanisms adapting bacteria to life in digestive tract show a physiological and clinical significance. Stress tolerance is very desirable in the case of symbiotic and probiotic species enabling them maintenance in the gastrointestinal tract. On the contrary knowledge about protective mechanisms in pathogenic species is very important to efficient fight against pathogens.

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Received: July 19, 2007
Accepted: August 15, 2007

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