Heat-shock protein protection

Heat-shock proteins (HSPs) are induced, in part, by denatured proteins produced during heat shock, ischemia and other stresses. They are found in all plant, yeast, bacterial and mammalian cells[1]. However, they are still relatively little studied in medically relevant fields, which includes the neurosciences. The HSP70 heat-shock protein, along with other chaperones, aids the restoration of the structure and function of the denatured proteins. Recent studies suggest that heat shock and viral overproduction of HSP70 protects brain cells *in vitro* against injuries that produce necrosis and some types of apoptosis. Overproduction of HSP70 *in vivo* protects the brain against injury produced by ischemia and prolonged seizures. Heat-shock proteins and glucose-regulated proteins (GRPs) provide molecular markers of specific types of cell stress. A particular HSP or GRP could protect against very specific types of injury or against a variety of pathological processes.

Virally delivered HSP70 protects the brain

A recent study by Yenari *et al.*[2] illustrates that HSP70 protects the brain against injury produced by ischemia and seizures. A defective herpes-simplex-virus vector, which expressed the gene encoding the heat-shock protein HSP70, was injected directly into rat brain. Neuronal survival, in the small numbers of neurons that were transfected, improved from 62.3 to 95.4% in the striatum following a one-hour middle-cerebral-artery occlusion, and improved from 22 to 64% in the hippocampus following kainic-acid administration. These experiments are limited by the fact that only a small percentage of a specific type of neuron can be transfected with this virus and no glial cells can be transfected. However, these data do suggest that overproduction of HSP70 can protect neurons against several types of injury and that stress proteins could be used to protect brain against a variety of acute destructive or chronic degenerative processes.

Heat-shock protein family members

There are many members of the HSP family and several families of stress-inducible proteins. The HSPs are defined physiologically by their ability to be induced by heat shock and, in molecular terms, by the presence of a functional heat-shock element in their promoter[3]. Heat-shock proteins serve as chaperones that bind to other proteins and regulate their conformation, regulate the proteins' movement across membranes or through organelles, or regulate the availability of a receptor or activity of an enzyme. Some of the known HSPs include ubiquitin, HSP10, HSP27, HSP32 (or HO-1), HSP47, HSP60, HSC70 (or HSC73), HSP70 (or HSP72), HSP90 and HSP100/105, most of the proteins are named according to their molecular weight.

HSC70 is the major chaperone found in normal cells

The gene encoding HSC70 is expressed constitutively and HSC70 is the most abundant HSP found in normal cells[3]. It binds to proteins as they are being synthesized on the ribosomes and prevents the formation of abnormal protein conformations[4]. HSC70 also serves as a chaperone, which regulates actin binding and the formation of clathrin-coated vesicles[5]. It is synthesized, to a modest degree, following focal and global cerebral ischemia[6] and could contribute to the phenomenon of ischemia-induced tolerance to ischemia[7].

HSP70 is the major inducible heat-shock protein

HSP70, also called HSP72, is the major inducible HSP found in all living cells[2]. Following heat shock, its synthesis increases to a point to where it becomes the most abundant single protein in a cell. HSP70 is synthesized in response to heat, heavy metals, toxins, ischemia and other stresses[5]. The expression of heat-shock genes is increased in response to any stress that produces denatured proteins, including heat shock[8] and ischemia[9]. The denatured proteins initiate the heat-shock response[10] possibly by binding HSP90, which results in the dissociation of HSP90 from heat-shock-factor proteins (HSFs). HSP90 normally binds to and suppresses the HSFs (Ref. 11). The dissociation of HSP90 from HSFs can lead to the phosphorylation of HSFs, perhaps by protein kinase C, which leads to HSF activation. The activated HSFs form trimers[12] that then bind to the promoter of the *Hsp70* gene to stimulate transcription[13] (Fig. 1). This leads to massive increases in levels of HSP70 protein and mRNA in the stressed cells. Once synthesized, HSP70 binds to denatured proteins[14]. After binding, HSP70 attempts to restore the tertiary structure and enzymatic activity of proteins in a cycle that is driven by ATP hydrolysis[15–17]. Denatured proteins, therefore, serve as a stimulus for the induction of the expression of *Hsp70*, and HSP70 in turn can restore protein structure and function.

Following focal cerebral ischemia mRNA encoding HSP70 is synthesized in

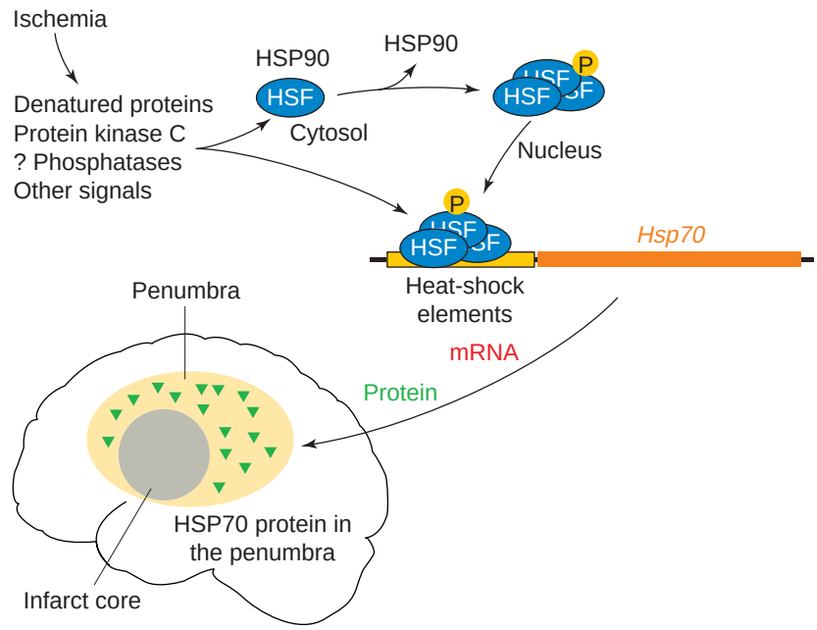

Fig. 1. ***The mechanism of heat-shock protein 70 (HSP70) production following focal ischemia in the brain.*** *Ischemia produces denatured proteins within cells. The denatured proteins activate heat-shock factors (HSFs), perhaps by promoting dissociation of HSP90 from the HSFs. The HSFs are phosphorylated, form a trimer and bind to heat-shock elements (HSEs) on heat-shock genes, including* Hsp70. *This activates HSP70 transcription although HSP70 mRNA might not be synthesized in the infarct where ATP is limited. Outside the infarction HSP70 protein is synthesized in glia and neurons in a region defined as the 'denatured protein' penumbra. HSP70, along with other chaperones, binds to denatured proteins to restore their structure and function and promotes survival of the glia and neurons. Abbreviation: P, phosphorylation.*

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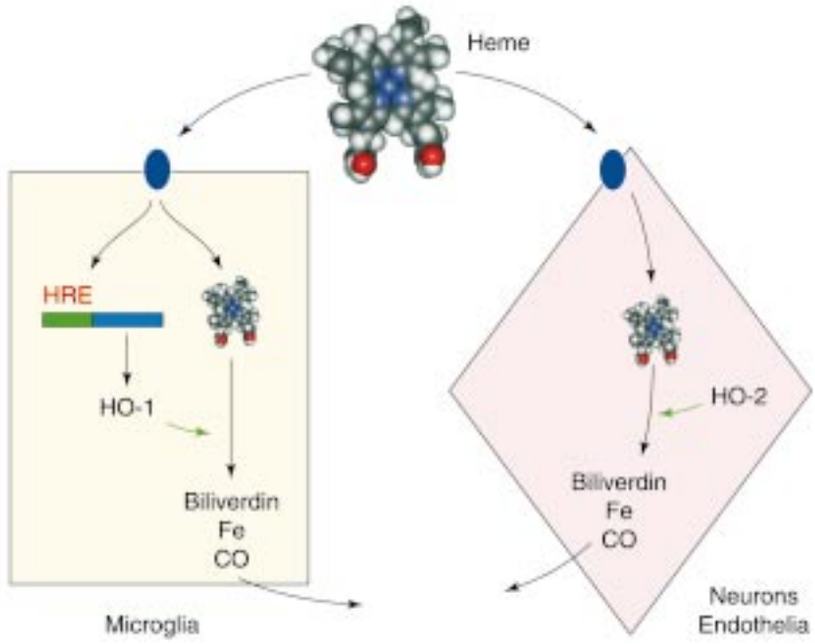

Fig. 2. ***The mobilization of heme derived from the extracellular space in microglia, neurons and endothelial cells.*** *Extracellular heme is derived from hemoglobin following hemorrhage or from heme proteins released from dying cells. Heme in microglia induced HO-1 mRNA transcription by activating a metal–heme-response element (HRE) on the promoter of the gene encoding heme oxygenase 1. Heme oxygenase 1 metabolizes heme to biliverdin, iron (Fe) and carbon monoxide (CO). Biliverdin reductase metabolizes biliverdin to bilirubin. Heme oxygenase 2 metabolizes heme to the same end products in neurons and endothelial cells. The fate of the iron released is uncertain, but it is probably bound by ferritin. Heme oxygenase 1 might be involved in the chaperoning of the iron to ferritin as HO-1 knockout mice do not process iron properly and deposit it abnormally in tissue. Abbreviations: HO-1, heme oxygenase 1; HO-2, heme oxygenase 2.*

most ischemic cells except in areas of very low blood flow. HSP70 protein is produced mainly in endothelial cells, in the core of infarcts in the cells that are most resistant to ischemia[18]. HSP70 protein is also found in glia at the edges of infarcts and in neurons outside the areas of infarction[19]. It has been suggested that this neuronal expression of *Hsp70* outside an infarct can be used to define one of several ischemic penumbras[20], with this expression indicating the zone of 'protein denaturation'[21] in areas of ischemia (Fig. 1).

HSP70 protects against necrosis and some types of apoptosis

A number of *in vitro* studies show that both heat shock and HSP overproduction protect CNS cells against both necrosis and apoptosis. Mild heat shock protects neurons against glutamate-mediated toxicity[22] and protects astrocytes against injury produced by lethal heat shock and lethal acidosis[23]. Transfection of glia with HSP70 protects them against heat shock and glucose or oxygen deprivation[24–26]. Transfection of cultured neurons with HSP70 protects them against otherwise lethal heat shock or simulated ischemia[24,27,28]. Although HSP70 protects cells in these studies, it cannot protect against overwhelming injury[23].

HSP70 overproduction can ameliorate apoptotic cell death produced by actinomycin, camptothecin, etoposide[29], ceramide[30] and radiation[31]. HSP70 and HSP90 do not protect against serum-withdrawal-induced apoptosis[32] and trigeminal neuronal cell death produced by NGF withdrawal[33]. HSP70 can inhibit caspase-3 activation caused by heat or ceramide and it can affect jun kinase and p38-kinase activation[30,34]. In addition, HSP70 binds to and modulates the function of BAG-1, the bcl-2-binding protein[35]. Viral delivery of a continuously active form of HSF to cultured neurons protects against heat shock and ischemia but not against a variety of apoptotic stimuli[36]. Hence, HSP70 appears to act upstream in some apoptotic cascades and can modulate some but not all types of apoptosis-related cell death at least in culture systems.

Heat-shock proteins also protect various types of cells *in vivo*. Heating an entire animal, which induces the synthesis of most HSPs, protects the retina against light-induced injury[37] and the brain against ischemia[38]. Overproduction of HSP70 in transgenic mice protects the heart from ischemia[39–41] and protects hippocampal neurons from focal ischemia[42]. The viral transfer of HSP70 (Ref. 2) probably worked in part because it delivered the HSP70 protein prior to the injury. Viral gene therapy, however, might not be practical for most pathological states in brain at present. Therefore, pharmacological methods of inducing HSP production are being sought. Though the noncompetitive NMDA-receptor antagonists, such as phencyclidine, induce heat-shock proteins in rodent brain[43], they produce undesirable psychotic side effects in humans. Thus, other drugs that also induce HSPs are being developed and tested for their ability to protect the brain.

Ubiquitin, HSP27, HSP47, HSP60, GRP75, GRP78 and GLUT1

Though HSP70 is produced in all brain cells, the expression of genes encoding other HSPs and GRPs is induced in specific cells or in specific organelles. Moreover, these stress proteins can be produced either by nonspecific stimuli, such as denatured proteins, or by very specific stimuli, such as low glucose levels (GRPs) or heme (heme oxygenase-1, HSP32).

Ubiquitin, one of the smallest of the HSPs, is expressed throughout brain and is produced in response to ischemia in all cells[44]. It is involved in the targeting of and chaperoning of proteins degraded in proteasomes[45], which include nuclear factor-κB, cyclins, HSFs, hypoxia-inducible factor, some apoptosis-related proteins, FOS, tumor necrosis factor (TNF), erythropoietin receptors and others[46].

Conversely, HSP27 is synthesized mainly in astrocytes by ischemia, administration of kainic acid and spreading depression[47]. It might chaperone cytoskeletal proteins, such as intermediate filaments, actin or glial fibrillary acidic protein, in astrocytes following stress[48]. Expression of the gene encoding HSP27 protects cells against lethal heat shock. It also protects against Fas–Apo-1, staurosporine, TNF (Ref. 49), camptothecin and etoposide-induced apoptotic cell death[29] as well as H_2O_2-induced necrosis[50]. HSP27 overproduction protects against simulated ischemia in cardiac myocytes[51] but does not protect against apoptotic death produced by *p53* expression[52].

HSP47, a collagen chaperone found in brain[53], is synthesized mainly in microglia by cerebral ischemia and subarachnoid hemorrhage[54,55]. HSP47 and other glial chaperones probably have a role in the cytoskeletal changes that occur when 'resting' microglia and astrocytes become 'activated.'

HSP60, glucose-regulated protein 75 (GRP75) and heat-shock protein 10 (HSP10)[56] chaperone proteins within mitochondria. GRP75 is synthesized in ischemic brain, mainly in neurons[57]. Another glucose-regulated protein, GRP78, is localized to the endoplasmic reticulum where it chaperones proteins through the endoplasmic reticulum[58]. It is also synthesized in response to ischemia and seizures[59]. GRP75 and GRP78, also called oxygen-regulated proteins (ORPs), are produced by low levels of glucose and oxygen, and Ca^{2+} ionophores. Included in this group is glucose transporter 1 (GLUT1), which is localized

to endothelial cells and astrocyte end feet, transports glucose into brain, and is synthesized in response to low levels of glucose and oxygen[60]. It protects brain cells against ischemia and seizures *in vivo* when overproduced using a viral vector[61]. Whether ubiquitin, HSP27, HSP47, HSP60, GRP75 or GRP78 will protect against ischemia, seizures and other injuries in whole brain has yet to be determined.

Heme oxygenase 1, the heme metabolizing HSP32

HSP32, also known as heme oxygenase 1 (HO-1), is synthesized mainly in microglia and is one of several related heme oxygenase proteins that metabolize heme to carbon monoxide, iron and biliverdin[62,63]. The gene encoding heme oxygenase 2 (HO-2) is constitutively expressed and found in neurons and endothelial cells[63] (Fig. 2). Heme oxygenase 1 and HO-2 regulate heme protein turnover, iron metabolism and oxidative stress[64], suppress oxidative stress through the action of the end product, bilirubin[62], and possibly contribute to carbon monoxide modulation of capillary endothelium[65] (Fig. 2). Heme oxygenase 1 is synthesized in response to heat shock, heme and oxidative stress[59]. It is not surprising that ischemia leads to the production of the HO-1 protein[66,67] but it is surprising, however, that cisterna magna injections of blood or hemoglobin increase the expression of the gene encoding HO-1 almost exclusively in microglia throughout brain[68]. This suggests that the microglia take up extracellular heme proteins following cell lysis or hemorrhage. Once in the microglia, heme induces the transcription of HO-1. Heme oxygenase 1 then metabolizes heme to biliverdin, CO and iron (Fig. 2). The iron released by HO-1 is bound by ferritin, perhaps via a HO-1 chaperone function[69]. Overproduction of HO-1 protects vessels against heme and hemoglobin-mediated injury[70]. Heme oxygenase 1 might also protect brain against blood- and hemoglobin-mediated injury.

Cell, organelle and stress specificity

What do these multi-faceted stress-protein responses tell us? Heat-shock proteins are synthesized in response to any stress that produces denatured proteins, whereas glucose-regulated proteins are synthesized as a result of low levels of glucose and oxygen. Expression of the gene encoding heme oxygenase 1 can be induced by heme. HSP70 is synthesized in all cells following ischemia and heat shock, as is ubiquitin. HSP27 is produced mainly in astrocytes whereas HO-1 (HSP32) and HSP47 are produced mainly in microglia. Expression of the gene encoding GLUT1 is upregulated mainly in endothelial cells and astrocyte end feet. GRP75, HSP60 and HSP10 are produced in mitochondria and GRP78 is synthesized in the endoplasmic reticulum. This cellular and organelle specificity demonstrates specific functions of these heat-shock or glucose-regulated proteins that could be unique for each stress, cell or organelle.

These stress- and cell- or organelle-specific responses provide molecular markers of the type and the location of injury in cells. This suggests that some stress proteins could protect against certain types of stress, such as heme, but will not protect against other types of stress. As HSP70 is produced in all cell types by a wide variety of injurious stimuli, this suggests that its modulation might have the greatest chance for protecting brain against a wide variety of pathological processes including ischemia and seizures.

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