Heat Shock Preconditioning and Eicosanoid Pathways Modulate Caspase 3-Like Activity in Superficially Injured Isolated Guinea Pig Gastric Mucosa

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Key Words

Heat shock protein · Gastric mucosa · Caspase

Abstract

Background: After superficial mucosal injury, the disturbed gastric epithelial continuity is restored by cellular migration. Caspase-3 is an enzyme responsible for the execution of stress-induced apoptosis. Interestingly, heat shock proteins (Hsp) including Hsp60 are capable of modulating caspase-3 activity. Interestingly, we have demonstrated that heat shock preconditioning upregulates Hsp synthesis and inhibits restitution and cell proliferation via mechanisms related to de novo protein synthesis and eicosanoid pathways, both of which are crucial in the regulation of apoptosis and gastric mucosal defense systems. **Aims:** To assess whether caspase-3 activity is affected by heat shock preconditioning and associated pharmacological modulations after standard superficial injury to allow development of cytoprotective strategies. **Methods:** Guinea pig gastric mucosae were mounted and perfused in paired Ussing chambers. After heat shock (HS) preconditioning (42°C) for 30 min, a superficial injury was induced (1.25 mol/l NaCl) followed by 3 h recovery. For mechanistic studies, the mucosa was exposed to 30 µmol/l arachidonic acid (AA) as a substrate for eicosanoid pathways, to 50 µmol/l quercetin

(Q) to inhibit lipoxygenases, to 50 µmol/l indomethacin (In) to inhibit cyclo-oxygenases, or to 150 µmol/l cycloheximide (CHX) to inhibit de novo protein synthesis. After the experiment, the mucosa was prepared for analysis of caspase-3 activity. Hsp60 expression was analyzed to monitor the induction of heat shock response. **Results:** HS upregulated Hsp60 expression, indicating induction of the heat shock response without an effect on basal caspase-3 activity. Superficial injury itself did not affect caspase-3 activity nor Hsp60 synthesis. In all the experiments, exposure to CHX abolished caspase-3 activity and Hsp60 synthesis. AA+Q increased, Q decreased, while In+AA and In+AA+Q abolished caspase-3 activity independent of alterations in Hsp60 synthesis. Upon exposure to In+Q, HS decreased caspase-3 activity and upregulated Hsp60 synthesis. **Conclusion:** Caspase-3 activity in isolated guinea pig gastric mucosa is regulated by mechanisms dependent on de novo protein synthesis and eicosanoid pathways but is not strictly related to Hsp synthesis. Upon modulation of the eicosanoid pathways, HS may be utilized to simultaneously decrease caspase-3 activity and increase Hsp synthesis. Modulations of the eicosanoid pathways may be utilized to reduce caspase-3 activity also upon normothermic conditions suggesting a novel mechanism by which caspase-3 is regulated in the gastric mucosa.

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Introduction

Gastric mucosa possesses the capability to repair by cell migration after superficial injury within the first few hours, i.e. restitution [1]. Preconditioning hyperthermia may be utilized to enhance the tolerance of the tissue to lethal injury via induction of synthesis of heat shock proteins (Hsp) [2, 3]. Hsp are stress-induced molecular chaperones, which repair denatured proteins and confer protection against apoptotic stimuli [4]. Hsp60 and Hsp70 protect against deep, ulcerative gastric injury [5–7], while their role in the repair of superficial injury remains unclear. In addition to changes in protein synthesis, heat shock induces release of arachidonic acid (AA) from plasma membranes and subsequent eicosanoid synthesis, i.e. activation of the AA pathways [8]. We have shown that heat shock preconditioning followed by normothermic recovery, inducing a modest 2- to 3-fold increase of Hsp70 levels, exerts a general inhibition on restitution and proliferation after superficial injury by a mechanism, that involves eicosanoid pathways and de novo protein synthesis [9–11].

Based on these results, we suggest that heat shock preconditioning might interfere with apoptotic cascades. In apoptotic cell death, the morphological and biochemical changes are a result of activation of a family of cysteine aspartic acid-specific proteases known as caspases [12–16]. Caspase activation is characterized by a sequential cascade involving upstream initiator caspases, which in turn activate downstream effector caspases. Initiator caspases are activated either by a mitochondrial stimulus [17, 18] or via death receptor-mediated pathway [19, 20]. The apoptotic signaling pathways converge to activation of effector caspase-3 resulting in changes characteristic of apoptotic cells [13]. Preconditioning heat shock has been shown to protect cells from apoptosis induced by subsequent hyperthermia via complete inhibition of effector caspases including caspase-3 [21]. Hsp60 closely interacts with caspase-3 and decrease of Hsp60 results in activation of caspase-3 and apoptosis [22–25]. However, these relationships vary in a tissue- and stimulus-dependent manner. In this study, we studied the effect of heat shock preconditioning and modulations of the eicosanoid pathways and de novo protein synthesis on Hsp60 and on the final apoptotic effector enzyme caspase-3 to clarify whether these could be modulated in superficially injured isolated guinea pig gastric mucosa.

Material and Methods

Perfusion of Gastric Mucosa

The experiments were performed as described previously [9–11]. Briefly, guinea pig stomach was removed and after seromuscular dissection, two equal halves of the fundic mucosae were mounted in Ussing chambers and perfused (37 °C). After a 75-min stabilization period (required for electrophysiological stabilization), the temperature of experiment tissue perfusion fluids was raised to 42 °C and maintained for 30 min. Subsequently, the temperature was normalized (37 °C) and the tissue was allowed to recover for 150 min. In experiments for the testing the effects of hyperthermia, the samples for caspase-3 and Hsp60 analysis were collected. Superficial injury was induced after the 150-min recovery period by exposure of the mucosal surface to 1.25 mol/l NaCl for 5 min. The experiment was continued thereafter for 180 min, which is required for the rapid epithelial restitution to occur [9–11] after which the samples for caspase-3 and Hsp60 analysis were collected. The experimental protocol was approved by the institutional committee for experimental animal use and research of Kuopio University.

Western Blot Analysis of Hsp60

The analysis was performed as described previously by our group [9, 10, 26, 27]. Briefly, the tissue samples were homogenized and sonicated and protein extracts (30 µg protein per lane) were electrophoresed on SDS-PAGE and transferred to a nitrocellulose membrane (Millipore, Bedford, Mass., USA) which was stained with Ponceau S solution (Sigma, St. Louis, Mo., USA), and checked for an even loading and transfer. As primary antibody, monoclonal antibody recognizing Hsp60 (SPA-806) (StressGen, Victoria, B.C., Canada) was used. As secondary antibody, horseradish peroxidase-conjugated anti-mouse antibody (Dako, Glostrup, Denmark) was used. The membranes were developed and quantitated using an image-analysis software (NIH-Image, USA). The optical densities were calibrated to the control values of native mucosae.

Detection of Caspase-3-Like Activity

The tissue samples were homogenized and sonicated similarly as in Western blot [9, 10, 26, 27]. The samples were centrifuged and supernatants collected. Caspase-3-like activity was measured using LANCE caspase-3 kit (Wallac, PerkinElmer, Turku, Finland) according to manufacturer's instructions. Briefly, protein concentrations were determined according to Bradford. Whole cell extracts (10 µg per well) were used. Time-resolved fluorescence quench (TR-FQA) caspase assay was used and the signals quantitated using Victor² multilabel counter (Wallac) at 37 °C after 180 min incubation. A substrate specific for caspase-3 and labeled with europium was used (Eu-CDEVDK-QSY 7). The signals obtained from the wells with samples were calibrated to signals obtained from the wells without samples but caspase-3 substrate and reaction buffer representing background. The caspase-3-like activity was verified using a specific inhibitor of caspase-3 (Ac-DEVD-CHO) (Pharmingen, San Diego, Calif., USA) at various concentrations (50–300 nM), and a sharp reduction of caspase-3-like activity to background level was observed at a final concentration of 100 nM.

Modulation of Protein Synthesis and Eicosanoid Pathways

Exogenous AA was added serosally and lumenally at the beginning of the experiment to yield a final concentration of 30 µmol/l, which has previously been shown to enhance heat shock response

Fig. 1. Effects of 30 min (42°C) heat shock and 150 min normothermic recovery (perfusion) (black bars), 30 min + 150 min normothermia 37°C (perfusion) (white bars), superficial injury by 1.25 mol/l NaCl (Injury) followed by 180 min recovery on gastric mucosal caspase-3-like activity in isolated and Ussing chamber perfused guinea pig gastric mucosa as analyzed by TR-FQA caspase assay. **A** Effect of inhibition of leukotriene and protein synthesis. **B** Effect of exogenous arachidonic acid. **C** Role of prostaglandins. **D** Role of prostaglandins

in the action of exogenous arachidonic acid. Pharmacological modulations: 50 µmol/l quercetin (Q), 150 µmol/l cycloheximide (CHX), 30 µmol/l arachidonic acid (AA) and 50 µmol/l indomethacin (In). Results are expressed as mean ± SEM (n = 5 per group). Wilcoxon's signed ranks test (* p < 0.05 heat shock preconditioning vs. normothermia). ANOVA: ^a p < 0.05 vs. heat shock preconditioning (injury); ^b p < 0.05 vs. normothermia (injury).

(Sigma) [28], stabilize gastric mucosa during heat shock and abolish the heat shock preconditioning-induced inhibition of restitution [9]. AA release [29] and metabolism via lipoxygenases were inhibited bilaterally with 50 µmol/l quercetin (Q) (Sigma) [30] which has also been shown to abolish the heat shock preconditioning-induced inhibition of restitution [9, 10]. AA metabolism via cyclooxygenases and prostaglandin synthesis was inhibited bilaterally with 50 µmol/l indomethacin (In) (Sigma) [31], which has also been shown to abolish the heat shock preconditioning-induced inhibition of restitution [9]. Total protein synthesis was inhibited bilaterally with 150 µmol/l cycloheximide (CHX) (Calbiochem, La Jolla, Calif., USA) [32], which has also been shown to abolish the heat shock preconditioning-induced inhibition of restitution [9].

Statistical Analysis

Wilcoxon's nonparametric signed rank test was used to analyze heat shock-induced effects between paired heat shocked experiment and normothermic control tissues. ANOVA with Bonferroni correction was used for analysis of drug effects by multiple comparisons. Data are presented as means ± SEM. A p value < 0.05 was considered significant.

Results

Effect of Inhibition of Leukotriene and Protein Synthesis in Heat Preconditioning

Drug Effects

Exposure to either Q or to CHX lowered caspase-3 activity as compared to corresponding unexposed control tissues (p < 0.05) (n = 5) (fig. 1A), whereas superficial injury did not have any effect (fig. 1A). Exposure to CHX lowered Hsp60 expression in heat shock preconditioned tissues compared to unexposed heat shock preconditioned tissues (p < 0.05) (n = 5) (fig. 2A).

Heat Effects

Heat shock preconditioning upregulated expression of Hsp60 in perfused tissues prior to injury (HS 2.3 ± 0.2 vs. NT 1.4 ± 0.1) (p < 0.05) (n = 5), in superficially injured tissues (HS 2.4 ± 0.2 vs. NT 1.3 ± 0.3) (p < 0.05) (n = 5)

Fig. 2. Effects of 30 min (42°C) heat shock and 150 min normothermic recovery (perfusion) (black bars), 30 min +150 min normothermia 37°C (perfusion) (white bars), superficial injury by 1.25 mol/l NaCl (Injury) followed by 180 min recovery on gastric mucosal expression of Hsp60 in isolated and Ussing chamber perfused guinea pig gastric mucosa as analyzed by Western blot with monoclonal antibodies. **A** Effect of inhibition of leukotriene and protein synthesis. **B** Effect of exogenous arachidonic acid. **C** Role of prostaglandins. **D** Role of prostaglandins in the action of exogenous arachidonic acid.

Pharmacological modulations: 50 µmol/l quercetin (Q), 150 µmol/l cycloheximide (CHX), 30 µmol/l arachidonic acid (AA) and 50 µmol/l indomethacin (In). Upper panel photomicrographs of original Western blots; lower panel bar graph. Results are expressed as mean ± SEM (n = 5 per group). Wilcoxon's signed ranks test (* p < 0.05 heat shock preconditioning vs. normothermia). ANOVA: ^a p < 0.05 vs. heat shock preconditioning (injury); ^b p < 0.05 vs. normothermia (injury).

and in tissues exposed to Q (HS 1.9 ± 0.2 vs. NT 0.9 ± 0.2) (p < 0.05) (n = 5) (fig. 2A) but not upon exposure to CHX. Upon these modulations, heat shock did not affect caspase-3 activity (fig. 1A).

Effect of Exogenous AA in Heat Preconditioning

Drug Effects

Exposure to AA+Q increased caspase-3 activity (p < 0.05) (n = 5), whereas exposure to AA+CHX decreased caspase-3 activity (p < 0.05) (n = 5) (fig. 1B). Exposure to AA+CHX lowered Hsp60 expression in heat shock preconditioned tissues compared to unexposed heat shock preconditioned tissues (p < 0.05) (n = 5) (fig. 2B).

Heat Effects

Hsp60 levels were elevated in heat shock preconditioned tissues upon exposure to AA (HS 2.7 ± 0.3 vs. NT 1.5 ± 0.2) (p < 0.05) (n = 5) and upon exposure to AA+Q (HS 2.4 ± 0.3 vs. NT 0.8 ± 0.2) (p < 0.05) (n = 5) (fig. 2B)

but not upon exposure to AA+CHX. No difference in caspase-3-like activities were found between heat shocked and normothermic tissues (fig. 1B).

Role of Prostaglandins in Heat Preconditioning

Drug Effects

Combination In+CHX decreased caspase-3 activity (p < 0.05) (n = 5) (fig. 1C). Exposure to indomethacin itself decreased levels of Hsp60 in the normothermic tissues compared to unexposed normothermic control tissues (p < 0.05) (n = 5) (fig. 2C). Exposure to In+CHX decreased both caspase-3-like activity and Hsp60 levels in both the heat shock preconditioned and normothermic tissues compared to corresponding unexposed control tissues (p < 0.05) (n = 5) (fig. 1C, 2C).

Heat Effects

In the tissues exposed to In+Q, heat shock decreased caspase-3-like activity as compared to normothermic con-

trol (HS 9.3 ± 1.6 vs. NT 20.2 ± 6.0) ($p < 0.05$) ($n = 5$) (fig. 1C), whereas no effect was observed upon exposure to In only or to In+CHX. Hsp60 levels were upregulated in heat shocked tissues exposed to In (HS 1.0 ± 0.2 vs. NT 0.4 ± 0.1) ($p < 0.05$) ($n = 5$) and in tissues exposed to In+Q (HS 1.7 ± 0.1 vs. NT 0.9 ± 0.1) ($p < 0.05$) ($n = 5$) but not in tissues exposed to In+CHX (fig. 2C).

Role of Prostaglandins and Leukotrienes in the Action of Exogenous AA

Drug Effects

Exposure to In+AA, In+AA+Q or In+AA+CHX decreased caspase-3-like activity compared to corresponding unexposed control tissues ($p < 0.05$) ($n = 5$) (fig. 1D).

Heat Effects

In the tissues exposed to In+AA+CHX, heat shock increased caspase-3-like activity slightly compared to normothermic control (HS 3.8 ± 0.5 vs. NT 3.3 ± 0.5) ($p < 0.05$) ($n = 5$) (fig. 1D).

Discussion

The major finding of this study is that upon modulations of eicosanoid pathways, heat shock preconditioning may be utilized to significantly decrease caspase-3 activity of superficially injured tissues within the immediate period of rapid epithelial repair, while simultaneously upregulating Hsp synthesis. Caspase-3 activity can also be pharmacologically modulated, independently of heat shock. In addition to our previous observations of Hsp70, heat shock upregulates synthesis of Hsp60, and this effect is sensitive to inhibition of protein synthesis and eicosanoid pathways.

In the present study, we showed that neither heat shock preconditioning nor superficial injury itself altered caspase-3 activity. The perfusion procedure used may account for the moderate caspase-3 activity found already in the uninjured perfused tissues. However, when comparing superficially injured restituted tissues, significant differences of caspase-3 activity were found depending on the pharmacological modulations. According to analysis with caspase-3 inhibitor Ac-DEVD-CHO, we suggest that the activity measured by the assay is solely due to caspase-3 activity. Quercetin lowered caspase-3 activity. Previously, we have shown that quercetin does not have effect on Hsp70 but it abolished the heat shock-induced inhibition of restitution [9]. There are several possible mechanisms by which quercetin could affect caspase-3

activity: inhibition of AA release [29], inhibition of leukotriene synthesis [30], direct effect of AA on caspase-3 [33] or antioxidant effect inhibiting mitochondrial reactive oxygen radical production [34]. It is possible that the perfusion procedure used in the present study exposes the tissue to a significant oxidative stress, which could be counteracted by quercetin.

Caspase-3 activity was sensitive to CHX, suggesting that de novo protein synthesis is required for caspase-3 activity. Blockade of protein synthesis was verified by abolished Hsp60 expression. Our results are supported by previous findings in guinea pig gastric mucosal cells in vitro, where CHX inhibited caspase-3 activity [35]. The effect of CHX on the integrity of the tissue is thereby considered beneficial since previously we have shown that it was also capable of blocking the inhibition of restitution and cell proliferation induced by heat shock preconditioning [9–11].

AA did not affect either caspase-3 activity or have additive effect on Hsp60 synthesis. Similarly, we have shown that the dose of AA used does not augment Hsp70 expression either alone or combined with quercetin [9]. Intracellular free AA is a substrate for enzymes generating eicosanoids essential for gastric mucosal defense [36, 37]. When AA metabolism was interfered by quercetin, caspase-3 activity was significantly increased. This is strikingly different from experiments where quercetin alone was used and exerted opposite effects. This may be due to perturbations of AA metabolism and turnover in plasma membranes, which are directly associated with regulation of caspase-3 activity [33, 38].

Caspase-3 activity was not sensitive to indomethacin. Previously, caspase-3 has been implicated in indomethacin-induced gastric epithelial apoptosis within 2 h from the exposure in rats in vivo [39]. Interestingly, when the tissues were exposed to both indomethacin and quercetin, heat shock decreased caspase-3 activity, whereas normothermic tissues retained basal activity. Therefore, upon exposure to quercetin, a mechanism involving indomethacin-sensitive pathways might be operative and regulate caspase-3 activity. Previously, preconditioning heat shock has been shown to protect against effector caspase, i.e. caspase-3 activation upon subsequent heat shock [21], and this function was considered to be mediated by Hsp70 chaperone activity [4, 21]. Previously, we have shown that upon these conditions, heat shock preconditioning results in 5- to 6-fold induction of Hsp70, which may explain the effect by its capability to inhibit caspase-3 [9]. This treatment is also capable of blocking the heat shock-induced inhibition of proliferation [11].

Hsp60 possesses similar capability to interact and inhibit caspase-3 as Hsp70 [22–25]. In the present study, only 1.7-fold induction of Hsp60 was observed, which suggests that these two chaperones are differentially regulated by perturbations of eicosanoid metabolism.

Interestingly, exposure to simultaneous indomethacin and arachidonic abolished caspase-3 activity, while neither AA nor indomethacin alone was found to affect it. Therefore, we suggest that prostaglandin synthesis or some other mechanism sensitive to indomethacin is a regulator of caspase-3 activity. Indomethacin is capable of blocking prostaglandin synthesis [31], releasing AA from plasma membrane [40] and it modulates intracellular calcium homeostasis [31]. Therefore, complex interactions between indomethacin and AA may account for the inhibition of caspase-3 activity. Interestingly, upon these conditions the levels of Hsp60 were independent of preconditioning.

The functions of Hsp70 and Hsp60 differ only slightly. Hsp70, the major inducible Hsp, is found mainly in the nucleus and cytosol and functions to minimize aggregation of newly synthesized proteins [41]. Hsp60 is found both in mitochondria and cytosol and facilitates the protein-folding process, unfolding and refolding of misfolded proteins [41]. With regards to caspase-interactions, these Hsp possess similar properties: Hsp70 is capable of rescuing cells from apoptosis after the activation of effector caspases [42], Hsp60 has been shown to inhibit caspase-3 [24].

Caspase-3 activity reflects apoptotic activity, which on the other hand has been shown to parallel the degree of deep necrotizing gastric mucosal injury induced by either ethanol or indomethacin in rats *in vivo* [39, 43, 44]. Although superficial injury itself did not affect caspase-3 activity, we suggest that treatments lowering caspase-3

activity while simultaneously upregulating Hsp synthesis, such as exposure to indomethacin and quercetin, might be suitable for cytoprotective strategies. These strategies might be utilized during gastric surgery, in which the gastric mucosa is susceptible to injury and delayed healing of anastomoses. However, it should be emphasized that superficial and deep mucosal injury differ in several aspects, such as the mechanism of repair and the time scale – the deep mucosal injury is always accompanied by inflammation and formation of scar tissue and heals within days or weeks, while restitution occurs in the absence of scar tissue within hours.

In conclusion, caspase-3 activity in superficially injured guinea pig gastric mucosa is sensitive to pharmacological modulation of eicosanoid pathways and *de novo* protein synthesis but not strictly related to Hsp synthesis. Heat shock preconditioning decreases caspase-3 activity of superficially injured tissues upon exposure to simultaneous quercetin and indomethacin while upregulating Hsp synthesis. The combined exposure to indomethacin and AA blocks caspase-3 activity also upon normothermia suggesting a novel mechanism by which caspase-3 activity is regulated in the gastric mucosa. We suggest that protocols which simultaneously downregulate caspase-3 activity and upregulate Hsp synthesis can be used to enhance the gastric mucosal defense.

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References

- Podolsky DK: Mucosal immunity and inflammation. V. Innate mechanisms of mucosal defense and repair: The best offense is a good defense. *Am J Physiol* 1999;277:G495–G499.
- Benjamin IJ, McMillan DR: Stress (heat shock) proteins: Molecular chaperones in cardiovascular biology and disease. *Circ Res* 1998;83:117–132.
- Buchner J: Supervising the fold: Functional principles of molecular chaperones. *FASEB J* 1996;10:10–19.
- Mosser DD, Caron AW, Bourget L, Meriin AB, Sherman MY, Morimoto RI, Massie B: The chaperone function of Hsp70 is required for protection against stress-induced apoptosis. *Mol Cell Biol* 2000;20:7146–7159.
- 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* 1991;101:161–166.
- 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* 1996;111:345–357.
- Itoh YH, Noguchi R: Pre-treatment with mild whole-body heating prevents gastric ulcer induced by restraint and water-immersion stress in rats. *Int J Hyperthermia* 2000;16:183–191.
- Calderwood SK, Bornstein B, Farnum EK, Stevenson MA: Heat shock stimulates the release of arachidonic acid and the synthesis of prostaglandins and leukotriene B₄ in mammalian cells. *J Cell Physiol* 1989;141:325–333.

- 9 Oksala NK, Paimela H, Alhava E: Heat-shock preconditioning affects restitution of isolated guinea pig gastric mucosa by an arachidonic acid and protein synthesis dependent mechanism. *Scand J Gastroenterol* 2002;37:1366–1373.
- 10 Oksala NK, Paimela H, Alhava E: Preconditioning hyperthermia inhibits restitution of isolated guinea pig gastric mucosa. *Eur Surg Res* 2002;34:339–345.
- 11 Oksala NK, Oksala A, Paavonen T, Alhava E, Paimela H: Heat shock preconditioning modulates proliferation and apoptosis after superficial injury in isolated guinea pig gastric mucosa via an eicosanoid and protein synthesis-dependent mechanism. *Apmis* 2003;111:497–506.
- 12 Cohen GM: Caspases: The executioners of apoptosis. *Biochem J* 1997;326:1–16.
- 13 Thornberry NA, Lazebnik Y: Caspases: Enemies within. *Science* 1998;281:1312–1316.
- 14 Nicholson DW: Caspase structure, proteolytic substrates and function during apoptotic cell death. *Cell Death Differ* 1999;6:1028–1042.
- 15 Hengartner MO: The biochemistry of apoptosis. *Nature* 2000;407:770–776.
- 16 Earnshaw WC, Martins LM, Kaufmann SH: Mammalian caspases: Structure, activation, substrates and functions during apoptosis. *Annu Rev Biochem* 1999;68:383–424.
- 17 Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X: Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 1997;91:479–489.
- 18 Zou H, Henzel WJ, Liu X, Lutschg A, Wang X: Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome c-dependent activation of caspase-3. *Cell* 1997;90:405–413.
- 19 Boldin MP, Goncharov TM, Goltsev YV, Wallach D: Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. *Cell* 1996;85:803–815.
- 20 Muzio M, Chinnaiyan AM, Kischkel FC, O'Rourke K, Shevchenko A, Ni J, Scaffidi C, Bretz JD, Zhang M, Gentz R, Mann M, Krammer PH, Peter ME, Dixit VM: FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex. *Cell* 1996;85:817–827.
- 21 Mosser DD, Caron AW, Bourget L, Denis-Larose C, Massie B: Role of the human heat shock protein hsp70 in protection against stress-induced apoptosis. *Mol Cell Biol* 1997;17:5317–5327.
- 22 Xanthoudakis S, Roy S, Rasper D, Hennessey T, Aubin Y, Cassady R, Tawa P, Ruel R, Rosen A, Nicholson DW: Hsp60 accelerates the maturation of pro-caspase-3 by upstream activator proteases during apoptosis. *EMBO J* 1999;18:2049–2056.
- 23 Samali A, Cai J, Zhivotovsky B, Jones DP, Orrenius S: Presence of a pre-apoptotic complex of pro-caspase-3, Hsp60 and Hsp10 in the mitochondrial fraction of Jurkat cells. *EMBO J* 1999;18:2040–2048.
- 24 Gupta S, Knowlton AA: Cytosolic heat shock protein 60, hypoxia and apoptosis. *Circulation* 2002;106:2727–2733.
- 25 Kirchhoff SR, Gupta S, Knowlton AA: Cytosolic heat shock protein 60, apoptosis and myocardial injury. *Circulation* 2002;105:2899–2904.
- 26 Oksala NK, Kaarniranta K, Tenhunen JJ, Tiisonen R, Heino A, Sistonen L, Paimela H, Alhava E: Reperfusion but not acute ischemia in pig small intestine induces transcriptionally mediated heat shock response in situ. *Eur Surg Res* 2002;34:397–404.
- 27 Kaarniranta K, Oksala N, Karjalainen HM, Suuronen T, Sistonen L, Helminen HJ, Salminen A, Lammi MJ: Neuronal cells show regulatory differences in the Hsp70 gene response. *Brain Res Mol Brain Res* 2002;101:136–140.
- 28 Jurivich DA, Sistonen L, Sarge KD, Morimoto RI: Arachidonate is a potent modulator of human heat shock gene transcription. *Proc Natl Acad Sci USA* 1994;91:2280–2284.
- 29 Fawzy AA, Vishwanath BS, Franson RC: Inhibition of human non-pancreatic phospholipases A₂ by retinoids and flavonoids. Mechanism of action. *Agents Actions* 1988;25:394–400.
- 30 Kim HP, Mani I, Iversen L, Ziboh VA: Effects of naturally-occurring flavonoids and biflavonoids on epidermal cyclooxygenase and lipoxygenase from guinea-pigs. *Prostaglandins Leukot Essent Fatty Acids* 1998;58:17–24.
- 31 Kokoska ER, Smith GS, Deshpande Y, Wolff AB, Miller TA: Indomethacin increases susceptibility to injury in human gastric cells independent of PG synthesis inhibition. *Am J Physiol* 1998;275:G620–G628.
- 32 Bhowmik A, Paimela H, Joutsu T, Alanko T, Paavonen T, Saksela O, Kivilaakso E: Induction of proliferation in isolated guinea pig gastric epithelium during restitution after superficial injury. *Dig Dis Sci* 1998;43:1507–1512.
- 33 Cao Y, Pearman AT, Zimmerman GA, McIntyre TM, Prescott SM: Intracellular unesterified arachidonic acid signals apoptosis. *Proc Natl Acad Sci USA* 2000;97:11280–11285.
- 34 Feng Q, Kumagai T, Torii Y, Nakamura Y, Osawa T, Uchida K: Anticarcinogenic antioxidants as inhibitors against intracellular oxidative stress. *Free Radic Res* 2001;35:779–88.
- 35 Johal K, Hanson PJ: Opposite effects of flurbiprofen and the nitroxybutyl ester of flurbiprofen on apoptosis in cultured guinea-pig gastric mucous cells. *Br J Pharmacol* 2000;130:811–818.
- 36 Takahashi S, Takeuchi K, Okabe S: EP4 receptor mediation of prostaglandin E₂-stimulated mucus secretion by rabbit gastric epithelial cells. *Biochem Pharmacol* 1999;58:1997–2002.
- 37 Takeuchi K, Ukawa H, Furukawa O, Kawachi S, Araki H, Sugimoto Y, Ishikawa A, Ushikubi F, Narumiya S: Prostaglandin E receptor subtypes involved in stimulation of gastroduodenal bicarbonate secretion in rats and mice. *J Physiol Pharmacol* 1999;50:155–167.
- 38 Surette ME, Winkler JD, Fonteh AN, Chilton FH: Relationship between arachidonate-phospholipid remodeling and apoptosis. *Biochemistry* 1996;35:9187–9196.
- 39 Slomiany BL, Piotrowski J, Slomiany A: Role of caspase-3 and nitric oxide synthase-2 in gastric mucosal injury induced by indomethacin: Effect of sucralfate. *J Physiol Pharmacol* 1999;50:3–16.
- 40 Levine L: Stimulated release of arachidonic acid from rat liver cells by celecoxib and indomethacin. *Prostaglandins Leukot Essent Fatty Acids* 2001;65:31–35.
- 41 Fink AL: Chaperone-mediated protein folding. *Physiol Rev* 1999;79:425–449.
- 42 Jaattela M, Wissing D, Kokholm K, Kallunki T, Egeblad M: Hsp70 exerts its anti-apoptotic function downstream of caspase-3-like proteases. *EMBO J* 1998;17:6124–6134.
- 43 Piotrowski J, Piotrowski E, Skrodzka D, Slomiany A, Slomiany BL: Gastric mucosal apoptosis induced by ethanol: Effect of antiulcer agents. *Biochem Mol Biol Int* 1997;42:247–254.
- 44 Slomiany BL, Piotrowski J, Slomiany A: Induction of tumor necrosis factor- α and apoptosis in gastric mucosal injury by indomethacin: Effect of omeprazole and ebrotidine. *Scand J Gastroenterol* 1997;32:638–642.