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ORIGINAL ARTICLE

Histamine modulates the cellular stress response in yeast

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Abstract The cellular stress response is a universal protective reaction to adverse environmental or microenvironmental conditions, such as heat and drugs, associated in part with the highly conserved heat shock proteins (HSPs). Histamine is a key inflammatory mediator derived from L-histidine that governs vital cellular processes beyond inflammation, while recent evidence implies additional actions in both prokaryotes and eukaryotes. This study explored the possible role of histamine in the heat shock response in yeast, an established experimental model for the pharmacological investigation of the cellular stress response. The response was evaluated by determining growth and viability of post-logarithmic phase grown yeast cultures after heat shock at 53°C for 30 min. Thermal preconditioning at 37°C for 2 h served as a positive control. The effect of histamine was investigated following long-term administration through the post-logarithmic phase of growth or short-term administration for 2 h prior to heat shock. Short-term treatment with 1 mM histamine resulted in de novo protein synthesis-dependent acquisition of thermotolerance, while lower doses or long-term administration of histamine failed to induce the heat-resistant phenotype. Preliminary investigation of HSP104, HSP70 and HSP60 expression by western blotting showed an increase of these proteins after thermal preconditioning. However, a differential HSP and tubulin expression appeared to underlie the response of yeast cells to histamine. In conclusion, histamine was capable of inducing the adaptive phenotype, while the contribution of HSPs and tubulin and the potential implications remain largely elusive.

Keywords Cellular stress response · Heat shock protein · Histamine · Tubulin · Yeast

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Introduction

The adaptive and protective universal cellular stress response is vital for cell survival and growth under adverse environmental or microenvironmental conditions (Tiligada et al. 2002). The transcriptional events of this evolutionary conserved multi-component phenotype are due mainly to heat shock transcription factor, which is regulated differently in various eukaryotic cells ranging from the yeast *Saccharomyces cerevisiae* to mammals (Truman et al. 2007). The heat shock transcription factor regulon is a critical determinant for the expression of the highly versatile heat shock proteins (HSPs) under a variety of chemical and physical stresses (Tiligada et al. 2002; Bösl et al. 2006; Tiligada 2006a, b) as well as for the transcription of non-*hsp* genes encoding proteins involved in diverse cellular processes such as detoxification, metabolism and cell wall integrity (Yamamoto et al. 2005; Truman et al. 2007). Many HSPs have housekeeping homeostatic functions such as their co-operative role in the formation and function of the eukaryotic cell cytoskeleton (Liang and MacRae 1997). Upon exposure to stress, their increased expression compromise the increases in partially unfolded proteins, the HSP70 family being an example (Kregel 2002). On the other hand, HSP104 is vital during acquisition of thermotolerance and allows cells to survive severe heat treatment frequently in co-operation with the HSP70 chaperones (Bösl et al. 2006).

The conservation of the basic cellular and molecular mechanisms from yeasts to higher eukaryotes made the yeast *S. cerevisiae* an established model for the investigation of the cellular stress response (Mager and Ferreira 1993; Miligkos et al. 2000; Tiligada et al. 2002). Like any other cell, yeasts often encounter and adapt to different types of environmental and microenvironmental stress and produce or assimilate biologically active molecules including histamine (Besançon et al. 1992; Gardini et al. 2006). In higher eukaryotes, most studies relating cellular stress response components to pathophysiology have focused on the implications in cardiovascular disease (Pantos et al. 2006), cancer and anticancer therapy (Miligkos et al. 2000; Papamichael et al. 2006; Tiligada 2006a, b; Tiligada et al. 2006). However, relatively limited data are available on the cross-talk between inflammatory mediators and the cellular stress response (van Eden et al. 2007).

Histamine is a biogenic amine derived from L-histidine, with multiple activities in inflammation, host defence, neurotransmission, gastrointestinal functions and cell proliferation, largely mediated by four types of G-protein-coupled receptors, H_1, H_2, H_3 and H_4 (Akdis and Simons 2006; Zampeli and Tiligada 2009). Besides the well-established receptor-mediated actions, additional intracellular roles for histamine emerge for both prokaryotic (Kyriakidis et al. 2008; Kyriakidis and Tiligada 2009; Theodorou et al. 2009) and eukaryotic (Novák and Falus 1997; Rinnerthaler et al. 2006; Isik et al. 2009) organisms. Although limited data are available, there seems to be an interaction between histamine and components of the cellular stress response. Histamine contributes to HSP27 phosphorylation in endothelial cells (Santell et al. 1992), while mast cell degranulation and mediator release may be differentially affected by heat shock (Mortaz et al. 2005; Kakavas et al. 2006). Moreover, distinct histamine receptors may exert neuroprotective actions via interaction with HSPs in fish exposed to stressful conditions (Giusi et al. 2008).

The importance of histamine in governing vital cellular processes directed this investigation towards its potential role in the heat shock response in yeast. The reported data provide first evidence for an inductive function of histamine during the cellular stress response, HSP and tubulin expression possibly contributing to the modulation of this phenotype.

Materials and methods

Yeast strain and culture media

The budding yeast *S. cerevisiae* ATCC 2366, also referred to as *S. pastorianus*, was maintained on yeast agar (YEPD, containing in w/v: 0.3% yeast extract, 0.5% mycological peptone and 1% dextrose, supplemented with 1.5% bacteriological agar; Oxoid, UK).

Drugs and reagents

Histamine dichloride was obtained from SERVA (Germany). Cycloheximide and acid-washed 425–600 μm glass beads were purchased from Sigma (USA). Nitrocellulose membranes (Hybond™ ECL™) and Hyperfilm™ were obtained from Amersham (UK). Antibodies for HSP70 (SPA-810), HSP60 (SPA-808) and HSP104 (SPA-1040) and the second antibody for HSP104 and β-tubulin (SAB-300) were purchased from Stressgen Biotechnologies Corporation (Canada), the second antibody for HSP60 and HSP70 (NA931V) from Amersham (UK) and the β-tubulin primary antibody (ab15568) from Abcam (UK). All other chemicals were of analytical grade.

The heat shock response

A single 2-day-old colony was inoculated into 5 ml YEPD, incubated at 27°C for 2 h and subsequently cultured in YEPD at 27°C for 24 h through to the post-logarithmic phase of growth. Cells were then submitted to heat shock (HS) at 53°C for 30 min (Tiligada et al. 1999). Thermal preconditioning was performed by shifting cells to 37°C for 2 h before HS and served as positive control (Tiligada et al. 1999).

Histamine, at doses of 0.01–10 mM, which were below the minimum inhibitory concentration, was added to the cultures during thermal preconditioning, the 24 h incubation period (long-term administration) or 2 h prior to HS (short-term administration). Cycloheximide, at 0.35 mM, was added 2 h before or during HS alone or in combination with 1 mM histamine. Control cultures, in the absence of any agent, were included in all experiments. In all cases, the pH was 5.7–5.8.

Evaluation of cell viability and growth

After HS, aliquots (0.1 ml) of the cultures were appropriately diluted with full strength Ringer's solution containing 0.1% of the vital exclusion dye methylene blue. An aliquot was loaded onto a Neubauer hemocytometer and examined under the optical microscope at 400× magnification (Zeiss, Germany). Viability was determined by counting the blue-stained dead cells and their unstained viable counterparts, and expressed as the percentage of the viable cells in each culture (Tiligada et al. 1999). The time course of the histamine-induced effects was monitored by determining viability, following exposure to HS, every 30 min during the short-term treatment

and at 2, 6, 12 and 22 h during the long-term treatment. In addition, yeast viability after HS was determined following short-term histamine administration during the lag and the exponential phases, at 2 and 6 h of growth, respectively. Yeast cells were viable before exposure to HS independently of the treatment. Any phenotypic variations in the survival and/or growth following HS were assessed after incubation of 0.2 ml aliquots of selected cultures on yeast agar at 27°C for 48 h. Colony growth was examined both macroscopically, in terms of colony forming units (CFUs), and under the optical microscope at 400× magnification.

Total protein extraction

Post-logarithmic phase cultures were pelleted by centrifugation at 1,200×*g* for 5 min (Heraeus, Germany). Following washing in double-distilled H_2O, an equal to the pellet volume of acid-washed glass beads and 200 µl of ice cold extraction buffer containing 10% (v/v) glycerol, 0.5 mM dithiothreitol, 0.5 mM polymethylsulphonyl fluoride and 0.5 µg/ml leupeptin were added. Subsequently, cells were disrupted by vigorous mixing for 5 repeated cycles of 45 s mixing/30 s resting on ice. The resulting homogenate was centrifuged at 1,200×*g* for 5 min at 4°C. Total protein was quantified in the supernatant (Bradford 1976) and used for immunoblotting.

Western blotting

The total protein extracts were boiled for 5 min in Laemmli buffer, containing 5% (v/v) 2-mercaptoethanol. Aliquots of 30 µg were loaded onto a 10% (w/v) polyacrylamide gel and subjected to SDS-PAGE (Mini PROTEAN®, Bio-Rad, USA) at 200 V for approximately 45 min, followed by electrophoretic transfer to a nitrocellulose membrane (Hybond™, Mini PROTEAN®, Bio-Rad, USA) for 1.5 h at 100 V and 4°C using Towbin buffer. Subsequently, membranes were blocked by 5% (w/v) non-fat dry milk in TBST for 1 h at room temperature (RT). Blots were washed 3 times in TBST for 5 min and incubated overnight at 4°C with either 1:1,000 HSP70, 1:7,000 HSP60, 1:2,500 HSP104 or 1:1,000 *β*-tubulin antibody. Following 3× washing in TBST for 5 min, blots were exposed for 1 h at RT to horseradish peroxidase-conjugated second antibody at 1:10,000 for HSP60 and HSP70 and at 1:32,000 for HSP104 and *β*-tubulin. Blots were washed as above, incubated with ECL™ reagents and exposed to Hyperfilm™. Immunoblots were quantified using an AlphaScan Imaging Densitometer (Alpha Innotech, USA). Sample loadings were monitored by reversible staining with Ponceau S.

Statistical evaluation

Viability was expressed as mean ± SEM from 4–12 independent experiments. Statistical analyses were performed by non-parametric tests and ANOVA with Dunnett T3 or Scheffé post hoc analysis. Correlation was determined by the Spearman's non-parametric test. All tests were performed using SPSS v.13, they were two-sided and $P < 0.05$ was regarded as statistically significant.

Results

Effect of histamine on yeast viability and growth after HS

Yeast viability after HS in control cultures grown for 24 h in the absence of any agent was 34.7 ± 1.1%. Thermal preconditioning resulted in statistically significant induction of viability to 70.0 ± 3.5% versus control cultures ($P < 0.001$, Mann–Whitney test). In this case, no effect was observed upon histamine administration (Spearman's $r = -0.049$, $P > 0.9$), yeast viability ranging from 67.0 ± 3.0 to 70.5 ± 4.5% for all histamine concentrations studied ($P > 0.9$, ANOVA).

Short-term administration of histamine for 2 h prior to HS resulted in a biphasic effect with significant increases in yeast viability at 0.1 and 1 mM compared to control ($P < 0.05$, ANOVA), followed by decreases at higher concentrations of the amine (Fig. 1). Maximal increases in yeast viability were observed even in the first 30 min of histamine administration, viability remaining elevated for

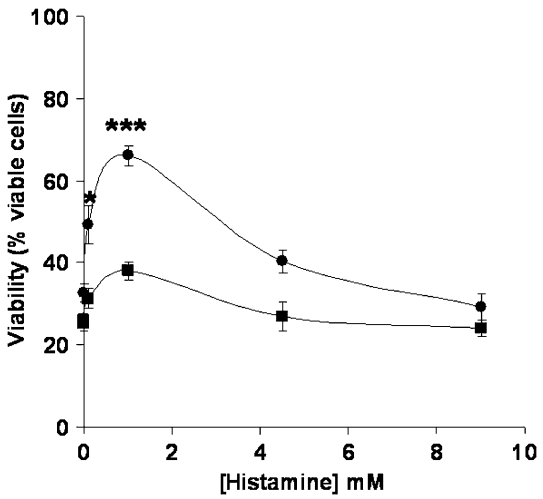

Fig. 1 Effect of histamine on cell viability after heat shock. Long-term (*filled squares*) and short-term (*filled circles*) histamine administration for 24 and 2 h prior to heat shock, respectively. Data are shown as mean ± SEM ($n = 4$–8). *$P < 0.05$, ***$P < 0.001$ versus respective untreated cultures (0)

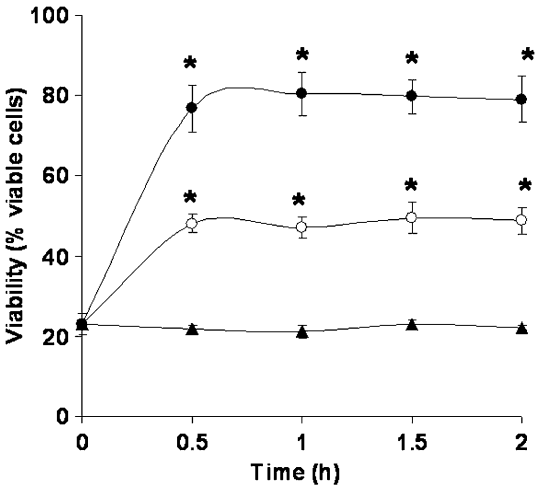

Fig. 2 Time course of cell viability after heat shock during the short-term administration of 0.1 mM (*open circles*) and 1 mM (*filled circles*) histamine and in control untreated yeast cultures (*filled triangles*). Data are shown as mean ± SEM ($n = 4$–8). *$P < 0.05$ versus respective untreated cultures (0)

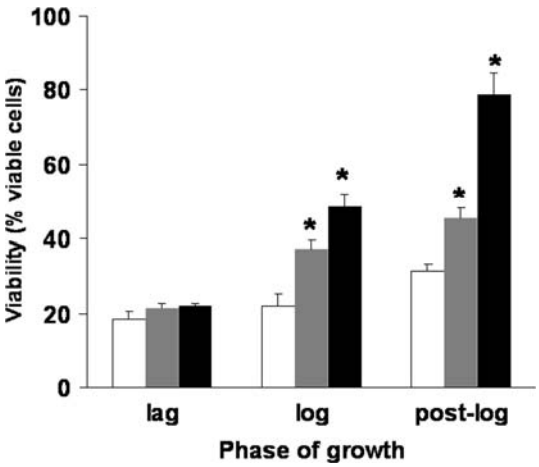

Fig. 3 Viability of yeast cells after heat shock following no treatment (*open bars*) or short-term administration of 0.1 mM (*grey bars*) and 1 mM (*closed bars*) histamine during the lag, exponential (log) and post-logarithmic (post-log) phases, at 2, 6 and 24 h of growth, respectively. Data are shown as mean ± SEM ($n = 3$–8). *$P < 0.05$ versus respective untreated cultures

the whole period of the 2 h short-term treatment (Fig. 2). Moreover, administration of 1 mM histamine during the HS resulted in significantly increased viability of 64.3 ± 5.2% compared to control ($P < 0.001$, ANOVA). Interestingly, significant increases ($P < 0.05$, ANOVA) in yeast viability were observed following short-term histamine administration during the exponential and post-logarithmic phases of growth, but not during the lag phase (Fig. 3). On the contrary, long-term histamine administration had no significant dose-related (Spearman's $r = 0.236$, $P > 0.1$; Fig. 1) or

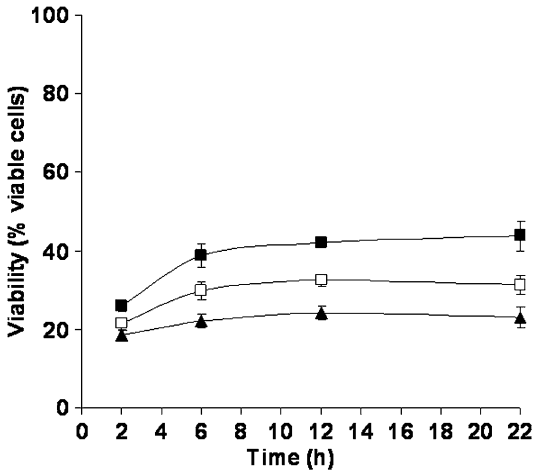

Fig. 4 Time course of cell viability after heat shock during the long-term administration of 0.1 mM (*open squares*) and 1 mM (*filled squares*) histamine and in control untreated yeast cultures (*filled triangles*). Data are shown as mean ± SEM ($n = 4$–8)

time-related ($P > 0.05$, ANOVA; Fig. 4) effects on yeast viability.

Formation of CFUs (Fig. 5a) and scattered single cells on the agar surface (Fig. 5b) were observed upon incubating untreated control yeast cultures on agar plates after HS. Increased number of CFUs (Fig. 5a′) and scattered cells on the agar surface (Fig. 5b′) were observed in samples that were submitted to thermal preconditioning at 37°C for 2 h prior to HS. Similarly, in accordance to the cell viability data (Fig. 1), short-term exposure to the adaptive histamine concentrations of 0.1 and 1 mM resulted in increased yeast growth, as shown by the larger CFU numbers compared to control and to the higher histamine concentrations of 10 mM (Fig. 5a) as well as by the presence of scattered cells on the agar surface following plating after HS (Fig. 5b). On the contrary, long-term exposure to all histamine concentrations resulted in relatively smaller CFU numbers (Fig. 5a′) and scattered cells on the agar surface (Fig. 5b′) compared to short-term treatment with histamine (Fig. 5a, b), in line with the cell viability data (Fig. 1).

Effect of de novo protein synthesis inhibition on the action of histamine on yeast viability after HS

The presence of the de novo protein synthesis inhibitor cycloheximide for 2 h before or during HS did not affect statistically significantly ($P > 0.05$, ANOVA) the viability of yeast cells after HS (Fig. 6). On the other hand, administration of cycloheximide resulted in the circumvention of the increased viability of yeast cells after HS acquired following short-term incubation in the presence of 1 mM histamine ($P < 0.05$, ANOVA; Fig. 6). Addition of

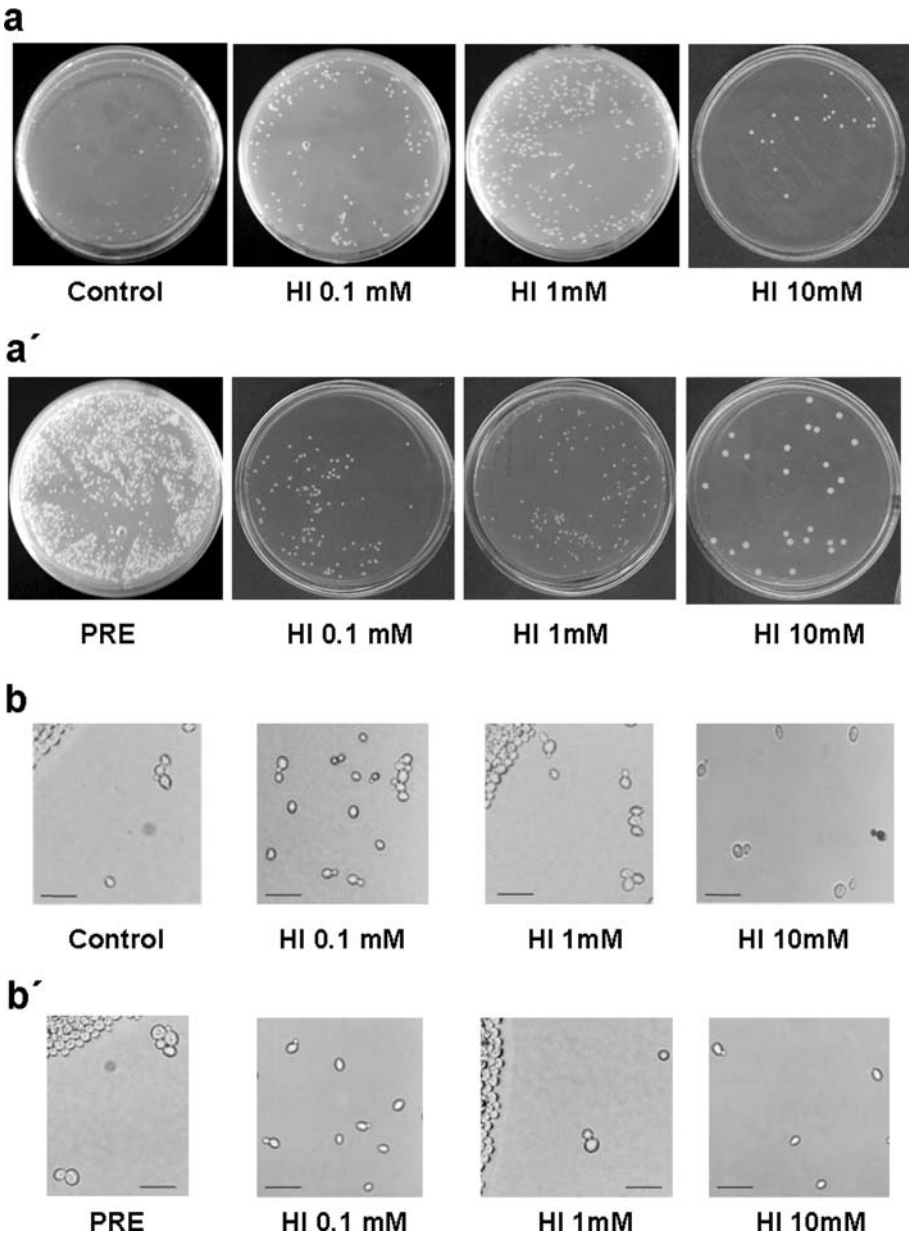

Fig. 5 **a**, **a′** Colonies and **b**, **b′** microscopic appearance after heat shock of untreated yeast cultures (control), after thermal preconditioning (PRE) and following **a**, **b** short-term and **a′**, **b′** long-term treatment with various concentrations of histamine (HI) for 2 and 24 h prior to heat shock, respectively. *Bar* represents 10 μm

cycloheximide during HS in cultures submitted to short-term conditioning with 1 mM histamine was unable to reverse the histamine-induced thermotolerance ($P > 0.05$, ANOVA; Fig. 6).

Effect of histamine on HSP expression

Preliminary experiments using *S. cerevisiae* total cell lysates demonstrated an up-regulation of HSP expression by about twofold following thermal preconditioning at 37°C (Table 1; Fig. 7). Although short-term administration of 1 mM histamine conferred significant thermotolerance to yeast cells (Fig. 1), it was unable to induce HSP expression to levels comparable to those obtained following thermal preconditioning (Table 1; Fig. 7). On the contrary, lower concentrations of histamine that were ineffective in inducing the thermotolerant phenotype (Fig. 1) showed a tendency to increase total cell lysate HSP levels (Table 1; Fig. 7). Interestingly, β-tubulin expression tended to decrease in thermotolerant yeast cultures (Table 1; Fig. 7).

Discussion

The data provided evidence for the ability of histamine to condition yeast cells and to induce the heat shock response. Contrary to the long-term histamine administration

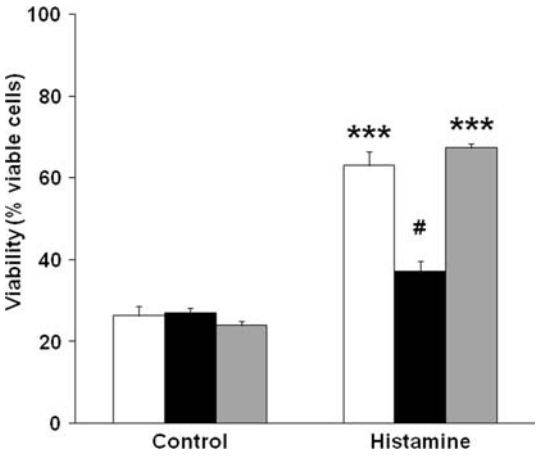

Fig. 6 Viability of yeast cells after heat shock following short-term administration of 1 mM histamine alone (*open bars*) or in combination with 0.35 mM cycloheximide either for 2 h before (*closed bars*) or during (*grey bars*) heat shock. Data are shown as mean ± SEM ($n = 4$–8). $^{***}P < 0.001$ versus untreated (control) cells. $^{\#}P < 0.05$ versus histamine alone

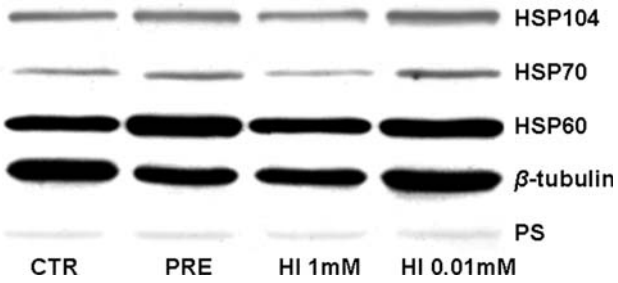

Fig. 7 Expression of heat shock proteins (HSPs) and β-tubulin in yeast cultures. Representative western blotting analyses of total cell lysates of control cultures (CTR), after thermal preconditioning (PRE) and following treatment with histamine (HI) for 2 h prior to heat shock. Band density was normalized against the band at 67 kDa obtained by reversible staining with Ponceau S (PS) in the same sample

(Figs. 1, 4), short-term treatment of exponentially and post-logarithmic phase growing cells with histamine before HS resulted to a dose-dependent induction of the thermotolerant phenotype (Figs. 1, 3). The observed early effect of histamine, upon short-term treatment in the post-logarithmic phase of growth, is supportive of a putative immediate protective action of histamine (Fig. 2), which seems to be in operation even during the potentially lethal HS.

The yeast *S. cerevisiae* is employed as a versatile and powerful model system for the investigation of the cellular stress response (Mager and Ferreira 1993; Miligkos et al. 2000; Stavrinidis et al. 2002; Yamamoto et al. 2005; Papamichael et al. 2006; Tiligada et al. 2006; Truman et al. 2007). Although *S. cerevisiae* lacks known homologues of histamine metabolic enzymes or receptors, the ability of histamine to induce the heat shock response dose dependently was indicative of the modulation of yet undefined signalling pathways by histamine in this unicellular organism. Along this line of research, recent reports provide evidence for histamine-induced activation of β-carbonic anhydrase, encoded by the Nce103 gene of *S. cerevisiae*, with activation constant of about 0.02 mM (Isik et al. 2009). Furthermore, in the prokaryotic *Escherichia coli*, histamine has been shown to affect a number of responses mediated by the AtoSC two-component system pointing towards a potential action of histamine in signal transduction processes in unicellular organisms (Kyriakidis et al. 2008; Kyriakidis and Tiligada 2009; Theodorou et al. 2009).

De novo protein synthesis appeared to be required for histamine to exert a conditioning effect in yeast cells, but not during survival under HS (Fig. 6). Histamine-induced thermotolerance was comparable to that observed following thermal preconditioning since yeast cells were not only viable but also capable of proliferating upon plating on agar after HS (Fig. 5). This pointed to the association of histamine to the adaptive phase of the heat shock response. De novo protein synthesis-dependent induction of thermotolerance in yeast has been observed after thermal preconditioning and following short-term exposure to a number of anticancer agents (Miligkos et al. 2000), prednisolone and the HSP90 inhibitor geldanamycin (Papamichael et al. 2006). On the other hand, the de novo protein synthesis-independent induction of thermotolerance by the phosphatase inhibitor sodium molybdate (Tiligada et al. 1999) and the inability of agents such as 17-β-estradiol to confer resistance to HS (Papamichael et al. 2006) provide evidence for stimulus-related induction of the response and

Table 1 Relative changes in the expression of heat shock proteins (HSPs) and β-tubulin determined by western blotting

Treatment	HSP104	HSP70	HSP60	β-Tubulin
Thermal preconditioning	1.96 ± 0.41	1.50 ± 0.02	1.65 ± 0.18	0.86 ± 0.02
[Histamine] = 1 mM	0.91 ± 0.21	1.10 ± 0.31	1.24 ± 0.14	0.96 ± 0.14
[Histamine] = 0.01 mM	1.27 ± 0.39	1.81 ± 0.33	1.59 ± 0.06	1.08 ± 0.02

Values represent the ratio of the densitometric values of bands containing the protein between the untreated control and the samples obtained after thermal preconditioning and following treatment with histamine for 2 h prior to heat shock. Values expressed are mean ± SEM from 2 to 4 independent experiments

for its differential modulation by intracellular signalling pathways.

In an attempt to identify a potential underlying mechanism in histamine-induced thermotolerance in yeast, preliminary determination of HSP expression was performed (Table 1). The induction of HSP expression during thermal preconditioning at 37°C (Fig. 7) was consistent with the critical role of HSP up-regulation in the cellular defence mechanisms against various stressors (Mager and Ferreira 1993; Yamamoto et al. 2005; Truman et al. 2007). In addition, a plethora of molecular alterations accompany the heat shock response in yeast, including amongst others, components of cell cycle control, RNAs and various genes associated with HSP induction (Shama et al. 1998), any of these being molecular candidate markers in the investigation of the stress response. Although at present it would be hard to suggest any underlying molecular mechanism, the fact that short-term administration resulted in an opposite effect of histamine on thermotolerance acquisition (Fig. 1) and HSP expression (Fig. 7) implies that non-HSP-dependent mechanisms may be involved in the histamine-induced thermotolerant phenotype in yeast. Experiments involving mutant strains for various HSPs could be used in the future in order to clarify their role in this first reported histamine-induced resistant phenotype in yeast. However, the tendency of increased HSP expression in response to low histamine concentrations, which however failed to induce heat resistance, and the trend of decreased β-tubulin expression that was observed in thermotolerant yeast cultures (Table 1) raise questions on the likely involvement of histamine in cytoskeletal changes during the stress response that are currently under investigation. Interestingly, the yeast orthologue of the mammalian histamine releasing factor/translationally controlled tumour protein has been proposed to be the housekeeping microtubule and mitochondria interacting protein, which appears to be highly expressed during active growth, but transcriptionally down-regulated in several kinds of stress situations (Rinnerthaler et al. 2006). Furthermore, the association between thermotolerance acquisition and the interplay of the imidazole ring and the positively charged hydrophobic backbone of histamine with diverse extracellular, membrane and intracellular components that would allow histamine to exert bulk effects on the cellular stress response is an intriguing hypothesis that cannot be excluded. It should be noted that rapid ion balancing has been suggested to contribute to cell survival during HS, while survival under mild stress could be coordinated by additional events (Tiligada et al. 1999; Vovou et al. 2004).

In conclusion, the findings provide first evidence on the preconditioning action of histamine during the cellular stress response and on its differential effects on the induction of thermotolerance and HSP expression in yeast. Since a variety of stressors are able to condition cells in order to survive under a subsequent severe shock through important cellular defence mechanisms, in addition to HSP expression, the hypothesis on the causal link of the development of stress tolerance to HSP expression remains elusive. Whether histamine elicits additional effects in eukaryotes, besides its well-established pleiotropic actions through its four types of receptors, with potential consequences in physiological and pathophysiological events is an interesting hypothesis open to investigation.

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