Influence of Oxidative Stress Induced by Cysteamine upon the Induction and Development of Thermotolerance in Chinese Hamster Ovary Cells

Rolf D. Issels, Susanne Bourier, Beatrice Böning, Gloria C. Li, John J. Mak, and Wolfgang Wilman

Abstract

Chinese hamster ovary cells exposed to the sulfhydryl compound cysteamine combined with heat treatment at 44°C developed thermotolerance within 8 h. After initial treatment either with 15 min cysteamine (0.4 mM) at 37°C immediately followed by 15 min heat at 44°C or with 15 min cysteamine (0.4 mM) at 44°C, the magnitude of thermotolerance developed was identical. The D₈₀ of the subsequent 44°C heat survival curves increased by factors of 8.9 and 7.9, respectively. The kinetics of thermotolerance induction and the time to reach the maximum of thermotolerance expression after combined cysteamine treatment at 44°C for 15 min was found to be comparable to the effects of 44°C treatment alone for 30 min. The synergistic effect of cysteamine with the conditioning heat treatment at 44°C was blocked by catalase (50 µg/ml).

INTRODUCTION

Cells from various organisms have been shown to develop a transient thermal resistance after exposure to elevated temperatures (1, 2). This phenomenon is called thermotolerance and is recognized as a potentially important factor influencing the biological response to hyperthermia in vitro and in vivo. In the past years, the time and temperature dependence for the induction, development, and decay of thermotolerance has been studied extensively (3-5). The induction of thermotolerance in mammalian cells can be achieved in two different ways: (a) by continuous incubation at temperatures between 41.5°C and 42.5°C for periods in excess of 3 or 4 h, the cells become thermotolerant during the time of heat treatment (6, 7); (b) pretreatments at temperatures between 38°C and 42.5°C render the cells resistant to a second treatment at 43°C or higher (3, 8). By fractional heat treatments at 43°C or higher, the cellular resistance to a second heat treatment is much more pronounced than by pretreatments below 42.5°C, but thermotolerance requires several h of incubation at 37°C to develop (1, 3, 9). Thermotolerance which developed during chronic heating or after acute heating seems to involve similar mechanisms. When cells became tolerant to chronic heating they have shown also to be tolerant to acute heating above 43°C and vice versa (10).

Despite the information about the temperature and temporal order of heat application to induce thermotolerance, there is very little known concerning the nature of the signal triggering this cellular response to heat. Also far from clear are the particular biochemical modifications within cells responsible for the cellular state of thermotolerance. Some studies have linked heat-induced cell killing and thermotolerance with perturbations of cellular GSH³ (11-13), a tripeptide involved in the maintenance of cellular oxidation-reduction potential (14). A possible role for oxygen radicals like superoxide in these processes has also been suggested (15).

An increase in the activity of superoxide dismutase in different types of cells was measured after exposure to hyperthermia which correlated with the development of thermotolerance. Heat also induces the synthesis of a specific set of HSPs in every organism thus far examined including cultured cells from a variety of animal sources (reviewed in Ref. 16). Development of transient thermotolerance following sublethal heat shock and its decay has been shown to correlate well with both the synthesis and the amount of HSPs (17-19). Cells treated with agents known to induce HSP synthesis also developed thermotolerance (20).

In this study, we exposed cells to the thiol compound cysteamine with or without heat in order to investigate its influence on the induction of thermotolerance for several reasons: (a) cysteamine generates activated oxygen species like superoxide and hydrogen peroxide (21, 22); (b) cysteamine leads to a rapid increase of cellular glutathione levels by a mechanism which is due to GSH biosynthesis (23); (c) thermosensitization by cysteamine of CHO cells has been reported (24, 25); and (d) this effect is dependent on the generation of activated oxygen species (26, 27).

MATERIALS AND METHODS

Cell Culture. CHO cells were routinely cultured in McCoy’s Medium 5A supplemented with 10% (v/v) newborn calf serum and 5% (v/v) fetal calf serum, penicillin (0.05 g/liter), streptomycin (0.05 g/liter), and neomycin sulfate (0.1 g/liter). Cells were maintained in exponential growth at 37°C in a 5% CO₂ atmosphere. Under these conditions, the population doubling time was approximately 14 h during the exponential growth phase and the plating efficiency was 80 to 90%. The cells were routinely subcultured every 2 or 3 days.

Heat and Drug Exposure. Twenty-four h prior to single or split dose treatments, exponentially growing cells were trypsinized (0.25% for 2 min) and counted, and dilutions of known cell numbers (10⁵-10⁶ cells/flask) were inoculated in 4 replicate T₂₅ flasks (Lux; Lab Tec) containing 4.5 ml of fresh medium (total volume). The flasks were placed in a
37°C incubator containing 5% CO₂ and air until treatment. Cysteamine (50 μl) was added directly to the warm medium (pH 7.4) to give a final concentration of 0.4 mM.

For initial treatments using cysteamine alone, after 30-, 60-, 90-, and 120-min drug exposure of cells at 37°C the medium was removed, and cells were washed twice with phosphate buffered saline (4.5 ml) and then covered with complete medium. Clonogenic cell survival of control cells treated under these conditions without drug was not affected (surviving fraction 95%).

For initial combined drug and heat treatments, cells were exposed to cysteamine (0.4 mM) at 37°C for 15 min and then washed as indicated above. An additional 15-min heat treatment at 44°C was given there-after (10 min) by submersing the sealed flasks in a circulating water bath (Umwelt-Thermostat W45/EB; Haake AG, Berlin, West Germany) at 44 ± 0.05°C. For comparison, cells were initially treated with a 15-min cysteamine exposure (0.4 mM) at 44°C. As a control for the effects of heat alone, cells were exposed for 15 or 30 min at 44°C. Temperature equilibration of the medium in the flasks at the cell surface with the water bath temperature (within 0.05°C) occurred in approximately 2.5 min. The medium pH ranged from 7.4 to 7.7 throughout the experiments. If not otherwise indicated, following all individual types of initial treatment, cells were maintained at 37°C for different time intervals (T₁ = 2, 6, 8, and 24 h) prior to the following heat treatment at 44°C given in all cases. Control (single dose) 44°C heat treatment survival curves were obtained each time pretreated cells were exposed to following heat treatments.

Clonogenic Cell Survival. After single or split dose treatments, the cells were incubated for 8-14 days for colony development. Following incubation, the colonies were rinsed with 0.9% NaCl solution, fixed, and stained with 20% ethanol containing 0.8% ammonium oxalate and 2% crystal violet. The fraction of treated cells giving rise to colonies (>50 cells/colony) was normalized to the fraction of control cells giving rise to colonies (plating efficiency). The surviving fraction was calculated after correction for cellular multiplicity (approximately 1.9), which was determined at the time of the first initial drug and/or heat treatment. The multiplicity corrected surviving fraction data, when plotted on log (surviving fraction) versus linear (dose) paper, yielded survival curves which are parallel. The fraction of treated cells giving rise to colonies (plating efficiency) and the plating efficiency at 37°C in methionine-free McCoy's Medium 5A, containing 1/100 normal levels of methionine. At the end of the labeling period, cells were treated with Cysteamine hydrochloride (β-mercaptoethylamine) was obtained from Sigma Chemical Co. and stored at 5°C in a desiccator. Stock solutions were freshly prepared by dissolving the chemical in cold Dulbecco's phosphate buffered saline solution, which had been previously gassed with N₂. The pH was adjusted to 7.2 to 7.4. Stock solutions of cysteamine were sterilized by filtering through a 0.22-μm Millipore or Schleicher & Schuell membrane and kept on ice until use (T = 1 h). Catalase from bovine liver (17,600 units/mg) was obtained from Sigma Chemical Co. Stock solution was freshly prepared in Dulbecco's solution and kept on ice.

RESULTS

Survival and Thermotolerance Response of CHO Cells Pretreated with Cysteamine and Heat at 44°C versus Heat at 44°C Alone. Initial experiments were performed to determine the influence of cysteamine upon the induction of thermotolerance by heat treatment at 44°C. The results of one typical experiment using different types of initial drug and heat treatments are given in Fig. 1. Cells were exposed to an initial treatment of 15 min cysteamine (0.4 mM) at 37°C immediately followed by a 15-min heat treatment at 44°C in the absence of drug (Treatment A). In comparison to this sequential application of drug and heat exposure, cells were simultaneously heated for 15 min at 44°C with cysteamine (0.4 mM) present during heating (Treatment B). Both types of combined drug and heat treatment reduced the surviving fraction of cells to a quite similar extent, which was 0.22 ± 0.02 and 0.09 ± 0.001, respectively. It should be noted that the surviving fraction of cells after 15 min cysteamine exposure at 37°C alone did not differ significantly from the plating efficiency of cells under these experimental conditions (see also "Materials and Methods"). A similar reduction in the surviving fraction (0.16 ± 0.01) as compared to the initial combined drug and heat treatments could be observed after a 30-min 44°C heat dose, which was used as an equivalent control for the effects of heat alone as a priming treatment (Treatment C).

Independent of the different conditioning treatments, cells became thermotolerant to a following heat treatment after a time interval (T₂) of 8 h at 37°C in all cases (see Fig. 1). The
second dose surviving fractions represent the results of one typical experiment. To facilitate comparison with the control curve, the second dose surviving fraction data have been normalized to a surviving fraction of 1.0. When a time interval of 12 or 24 h at 37°C separated the individual treatments ($T_i = 12$ or 24 h) there was no substantial change in the expression of thermotolerance (data not shown). For the time interval ($T_i = 8$ h), 2 to 5 independent experiments have been performed with the same qualitative results. The control and second dose $D_0$ values as well as the TTRs indicating the degree of thermotolerance for these repeated experiments are listed in Table 1. At $T_i = 8$ h, the magnitude of thermotolerance in terms of the calculated TTR values are similarly expressed after both types of combined treatments (TTR of 8.9 and 7.9, respectively) and not markedly different from the effect after 30 min heat at 44°C alone (TTR 9.3). The results further demonstrate that the induction of thermotolerance observed after cysteamine and 44°C heat treatment is independent of the temporal order of drug and heat application.

The influence of cysteamine combined with heat versus heat treatment alone upon the kinetics of thermotolerance induction in CHO cells was also investigated. The results of three independent experiments are shown in Fig. 2. Following the initial treatment with 15 min 0.4 mM cysteamine at 44°C, a single second heat dose (44°C, 45 min) was given after incubation of cells for 0 to 15 h at 37°C. By comparison with a priming treatment of 30 min at 44°C the kinetics of induction was similar (see Fig. 2, top). By addition of catalase (50 μg/ml) present during the combined 0.4 mM cysteamine treatment at 44°C, thermotolerance development was comparable with the effect of a priming treatment of 15 min heat at 44°C (see Fig. 2, bottom). The major difference in thermotolerance was observed at a 6-h time interval by comparison between the results shown in Fig. 2. At $T_i = 6$ h, the increase of thermal resistance to a second heat dose was less pronounced after 15 min cysteamine at 44°C and 30 min heat at 44°C (Fig. 2, top) compared to the effects of 15 min cysteamine at 44°C plus catalase and 15 min heat at 44°C alone (Fig. 2, bottom) given as priming treatments.

Therefore, the effect of cysteamine upon the conditioning heat treatment was further analyzed at this time interval. Fig. 3 shows the 44°C heat survival curves (0–120 min) of one typical experiment obtained at $T_i = 6$ h after the same initial priming treatments were given as described above. The control and second dose $D_0$ values of 3 repeated experiments as well as the TTRs indicating the degree of thermotolerance development at $T_i = 6$ h are compiled in Table 2. The expression of thermotolerance after treatment with 15 min cysteamine at 44°C or 30 min heat at 44°C was similar (TTRs of 6.6 and 5.5, respectively). By addition of catalase (50 μg/ml) immediately before the 15-min cysteamine treatment at 44°C, the TTR increased (10.2) and was similar to the TTR obtained after 15 min 44°C heat treatment alone (10.8).

The possible effect of catalase alone upon the priming heat treatments has also been checked in control (no cysteamine) experiments. By using either heat (44°C) plus catalase (50 μg/ml) or heat (44°C) alone as priming treatments, the $D_0$ values of the second heat survival curves were essentially identical (data not shown).

**Survival and Thermotolerance Response of CHO Cells Pretreated with Cysteamine at 37°C Alone.** Fig. 4 shows the survival data of CHO cells in one typical experiment after an initial exposure to 0.4 mM cysteamine for 2 h at 37°C followed by a heat treatment at 44°C. After a time interval of only 2 h incubation at 37°C in the absence of cysteamine ($T_i = 2$ h), cells became thermotolerant. The reduction of the surviving fraction by initial pretreatment with cysteamine was approximately 10 to 30% under these experimental conditions. Addition of catalase (50 μg/ml) prior to the addition of cysteamine and present only for 2 h during the initial drug exposure blocked the cytotoxic effects of cysteamine and thereby the induction of thermotolerance. Addition of superoxide dismutase (10 μg/ml) before cysteamine exposure could not block the induction of thermotolerance (data not shown).

When cells were exposed to 0.4 mM cysteamine at 37°C for different lengths of time ($T_i = \text{min, 37°C}$), the effect on thermotolerance was dependent upon the duration of the initial drug exposure. These results of repeated experiments are shown in Table 3. The $D_0$ values and TTRs were obtained by regression analysis of the exponential parts of the heat survival curves (for further details see "Materials and Methods").

Heat resistance acquired at $T_i = 2$ h by pretreatment with cysteamine at 37°C enhanced the $D_0$ of the 44°C control curves.

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**Table 1** Survival curve parameters ($D_0$, min) and thermotolerance ratio for 8-h time interval

<table>
<thead>
<tr>
<th>Type of pretreatment</th>
<th>No. of experiments</th>
<th>$D_0$ $\pm$ SD</th>
<th>Control $D_0$ $\pm$ SD</th>
<th>TTR $^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. 15 min, 37°C 0.4 mM cysteamine + 15 min, 44°C</td>
<td>3</td>
<td>51.1 ± 3.9 $^b$</td>
<td>5.7 ± 0.7</td>
<td>8.9</td>
</tr>
<tr>
<td>B. 15 min, 37°C 0.4 mM cysteamine</td>
<td>2</td>
<td>48.4 ± 3.8</td>
<td>6.1 ± 0.5</td>
<td>7.9</td>
</tr>
<tr>
<td>C. 30 min, 44°C</td>
<td>5</td>
<td>56.0 ± 3.2</td>
<td>6.0 ± 0.4</td>
<td>9.3</td>
</tr>
</tbody>
</table>

$^*$ For definition see "Materials and Methods."

Mean ± SD.
without catalase (Li). In all cases, after incubation of cells for 6 h at 37°C, graded
cysteamine at 44°C with catalase (50 μg/ml) present (A) and 15 min heat at 44°C
the initial pretreatment upon clonogenic cell survival are explained in the legend
second heat treatments of 44°C (0-120 min) were given at this time interval (7".
number of colonies of 4 to 6 replicate dishes; bars, SE.

Fig. 3. Exponential phase CHO cells were exposed (top) to 15 min 0.4 mM
cysteamine at 44°C (A) and 30 min heat at 44°C (B) or (bottom) 15 min 0.4 mM
cysteamine at 44°C with catalase (50 μg/ml) present (A) and 15 min heat at 44°C
without catalase (B). In all cases, after incubation of cells for 6 h at 37°C, graded
second heat treatments of 44°C (0-120 min) were given at this time interval (7".
points, mean number of colonies of 4 to 6 replicate dishes; bars, SE.

Table 2: Survival curve parameters (Do, min) and TTR for 6-h time interval

<table>
<thead>
<tr>
<th>Type of pretreatment</th>
<th>No. of experiments</th>
<th>Do</th>
<th>TTR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>5.8 ± 0.8b</td>
<td></td>
</tr>
<tr>
<td>15 min, 44°C</td>
<td>3</td>
<td>38.7 ± 3.2</td>
<td>6.6</td>
</tr>
<tr>
<td>+ 0.4 mM cysteamine</td>
<td>3</td>
<td>32.3 ± 2.1</td>
<td>5.5</td>
</tr>
<tr>
<td>30 min, 44°C</td>
<td>3</td>
<td>59.3 ± 7.2</td>
<td>10.2</td>
</tr>
<tr>
<td>15 min, 44°C + 0.4 mM cysteamine + catalase</td>
<td>3</td>
<td>62.8 ± 3.8</td>
<td>10.8</td>
</tr>
</tbody>
</table>

* For definition see "Materials and Methods."

b Mean ± SD.

c Catalase (50 μg/ml medium) was added immediately before cysteamine exposure.

by factors of 1.5, 1.6, 2.2, and 2.5 for t1 = 30, 60, 90, and 120
min. This increase in heat resistance observed at T1 = 2 h was
much less pronounced after further incubation (T2 = 8 h) of
cells in the absence of drug prior to second treatment (data not
shown). The Do of the second heat survival curves of cysteamine
pretreated cells (t1 = 120 min, 37°C) in the presence of catalase
was not significantly different from the Do of the 44°C control
curves (see Table 3). Also reincubation of cells with catalase
(50 μg/ml) alone at 37°C prior to 44°C heat treatment did not
affect the clonogenic cell survival (Do of 5.2) compared to cells
only heated at 44°C (Do of 5.7).

HSP Response of CHO Cells Pretreated with Cysteamine and Heat versus Heat Alone. As shown in Fig. 5, exposure of CHO
cells either to cysteamine combined with heat (44°C) or heat
alone (for details see legend of Fig. 5) induced a strong and
identical pattern of HSPs with molecular weights of 110,000,
87,000, and 70,000/68. Moreover, no major difference in the
induction kinetics of HSP was observed after both types of
pretreatment. In both cases, the autoradiographs of labeled
protein showed an increased rate of HSP synthesis within 6 to
8 h after initial treatments.

HSP Response of CHO Cells Pretreated with Cysteamine at 37°C Alone. Following exposure to graded doses of 0.4 mM
Cysteamine at 37°C, we also found an enhanced synthesis of
HSP in CHO cells. As compared with the densitometer tracing
of control cells, the profiles of protein synthesis after 30, 60,
90, and 120 min of cysteamine treated cells show a slight but
significant increase in the rate of the M, 70,000 and M, 87,000
protein (see Fig. 6).

For example, 8 h after 90-min cysteamine treatment at 37°C,
the relative rate of synthesis of the M, 70,000 protein was
increased to 160% of the control value. Catalase suppressed the
enhanced synthesis of this protein; if catalase (50 μg/ml) was
present during the cysteamine treatment, the relative rate of
synthesis of the M, 70,000 protein was almost identical to the
control value. Also no increase in HSP synthesis could be
observed between 2 and 6 h after cysteamine treatment.

DISCUSSION

The autoxidation of sulfhydryl compounds like cysteamine
in the presence of oxygen involves the univalent reduction of

Table 3: Survival curve parameters (Do, min) and TTR for 2-h time interval

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Time (min)</th>
<th>No. of experiments</th>
<th>Do</th>
<th>TTR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>0</td>
<td>7</td>
<td>5.7 ± 0.3b</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td>8.5 ± 1.3</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>2</td>
<td>9.0 ± 0.9</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>2</td>
<td>12.5 ± 1.4</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>4</td>
<td>14.4 ± 2.3</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>120 + catalase</td>
<td>5</td>
<td>5.8 ± 0.7</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

* For definition see "Materials and Methods."

b Mean ± SD.

c Catalase (50 μg/ml medium) was added immediately before cysteamine exposure.
INFLUENCE OF OXIDATIVE STRESS BY CYSTEAMINE UPON THERMOTOLERANCE

Fig. 5. Autoradiogram of a sodium dodecyl sulfate-polyacrylamide slab of [35S]methionine-labeled proteins from cysteamine (0.4 mM) plus heat (44°C) and heat (44°C) alone treated CHO cells. Cells were exposed to cysteamine (37°C, 30 min) followed by 30 min heat at 44°C (← CYST + 44°C) or exposed for 30 min at 44°C alone (← 44°C). After each type of treatment, cells were pulse labeled with [35S]methionine for 1 h at 37°C. C, untreated control; 0–24 h, time (h) of incubation before labeling. A, actin (M, 43,000). Major HSP induction with M, 110,000, 87,000, 70,000/68 indicated by arrowheads (top to bottom).

Fig. 6. Densitometer tracing showing ability of cells to enhance the synthesis of M, 87,000 and M, 70,000 proteins following exposure with cysteamine. CHO cells were exposed to 0.4 mM cysteamine at 37°C for 30, 60, 90, or 120 min or 90 min plus catalase (50 μg/ml). The profiles of protein synthesis were examined 8 h after treatment. Molecular weight (×10^-3) actin (A) are shown. HSP induction with M, 87,000 and 70,000 indicated by arrows.

Oxygen to (O_2^-) superoxide and the formation of (H_2O_2) hydrogen peroxide by a consequent dismutation reaction (21, 27). Both of these activated oxygen species are freely diffusible across cellular membranes. We have reported previously that the thermosensitizing effect of cysteamine in CHO cells is based on oxidative stress induced by the sulfhydryl compound at low concentrations (25, 26). We also found a rapid increase of intracellular GSH after exposure of CHO cells with cysteamine at concentrations known to generate activated oxygen species (23).

In the present study, we describe a substantial effect of cysteamine combined with a fixed heat dose (15 min, 44°C) upon the magnitude of thermotolerance and the time to reach its maximum of expression. By comparison with an equivalent heat dose alone, the kinetics of thermotolerance development was similar to that observed after a priming treatment of 30 min heat at 44°C.

The thermotolerance induced within cells has been shown by several studies to depend on the survival level following the priming treatment, irrespective of the treatment temperature and the heating time used to obtain this survival level (3, 9, 29, 30). According to these results, the synergistic effect of cysteamine upon heat conditioning was found to be independent of the temporal order of drug and heat application used in our studies and only dependent on similar reduction in cell survival achieved by both pretreatments. On the basis of our previously published results on the heat interaction with cysteamine, we used a surviving fraction only greater than 10^-2 for combined treatments since at this level the mechanisms of interaction have shown to be at least additive (25). It is interesting to note with regard to the mechanisms that cysteamine exposure at 37°C immediately followed by heat in the absence of drug leads to the same effect upon the priming heat dose as cysteamine exposure at 44°C (see Fig. 1 and Table 1).

From our results it seems to be most likely that the effect of cysteamine on heat conditioning is based on oxidative stress, especially the generation of H_2O_2. In the experiments using catalase during combined drug and heat exposure, the enhancement of the initial priming treatment by cysteamine was completely reduced to the effect of heat alone. Although the rate for thermotolerance induction was not modified when cells were pretreated in the presence of catalase, the time interval necessary to obtain thermotolerance after cysteamine and heat...
exposure was markedly changed (see Fig. 2). The effect of catalase is most probably explained by scavenging H$_2$O$_2$ and thereby blocking the sensitizing effect of cysteamine upon the following heat exposure in these experiments. In a comprehensive study, Majima and Gerweck (4) showed a substantial sparing effect due to dose fractionation of the initial heat treatment. By comparison with their results, we found that the time needed to reach expression of thermotolerance increased similarly with decreasing survival levels after priming treatments. Inhibition of the cysteamine effect by catalase was substantial at $T_c = 6$ h, where also the sparing effect due to either heat treatment alone or combined drug and heat treatment was most pronounced.

The observed dose-dependent increase in the heat resistance of CHO cells pretreated with cysteamine at 37°C is especially interesting with regard to the mechanisms of thermotolerance induction. It has been reported previously that Salmonella typhimurium pretreated with H$_2$O$_2$ become resistant to killing by heat (31). This adaptation was accompanied by the enhanced synthesis of a set of proteins, three of which were also induced by heat shock. More recently, a slight but significant increase in resistance to 43°C heat treatment was found to be induced in CHO cells following H$_2$O$_2$ pretreatment (32). The data strongly suggest that activated oxygen species like H$_2$O$_2$ play an important role in the molecular process triggering thermotolerance. Our results support this idea, since the addition of catalase blocked the induction of heat resistance by cysteamine at 37°C. However, with regard to the mechanism, the rapid elevation of intracellular GSH by thiols (23) which parallels the thermosensitizing and cytotoxic effects of such compounds (25, 26) might be equally important. GSH elevation during the induction of thermotolerance has been reported by Mitchell et al. (12, 13). Also, reduction of its development and decrease in HSP synthesis could be observed by GSH depletion and prevention of GSH biosynthesis prior to heating (11, 12). In the case of cysteamine, it seems possible that the interaction of elevated GSH content with oxygen species like H$_2$O$_2$ after cysteamine treatment causes significant perturbation of the intracellular GSH system and thereby triggering of the induction of thermotolerance. The formation of glutathione disulfide following cysteamine treatment could be an important signal for further modulation of protein synthesis similar to the substantial effects of glutathione disulfide on the initiation of protein synthesis in rabbit reticulocytes (33). The kinetics of cellular GSH increase and its decay after cysteamine treatment (23) is similar to the observed induction of heat resistance in the study which occurred within 2 h after cysteamine treatment. These results further support the idea that the induced increase in GSH may contribute a major effect in the signal for the induction of heat resistance by cysteamine. The interaction of GSH and H$_2$O$_2$ in this mechanism might also explain why the kinetics of heat resistance after H$_2$O$_2$ treatment alone differed significantly from our results with cysteamine (32). Our observation that cysteamine exposure at 37°C also leads to slight increase in the synthesis of HSP (e.g., $M$, 70,000 and $M$, 87,000) allows us to study in more detail the modulation of HSP synthesis by oxidative stress under these conditions. It seems most likely that the reported induction of HSP in various mammalian cells by sulphydryl reagents, transition series metals, chelating agents (34, 35), and superoxide (36) might be based on a common mechanism via oxidative stress. Such agents as cysteamine may act as components of a Fenton type reaction (37), a chemical process which generates highly reactive oxygen species in a metal catalyzed reaction.

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