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HEAT SHOCK INDUCTION OF HEME OXYGENASE mRNA IN HUMAN HEP 3B HEPATOMA CELLS

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Heat shock treatment of human Hep 3B hepatoma cells led to the induction of mRNA for microsomal heme oxygenase. The maximum induction of heme oxygenase mRNA (5↔7-fold) was observed with treatment of cells at 43.5°C, for 60 min. The heat-mediated induction of heme oxygenase mRNA was blocked by simultaneous treatment of cells with actinomycin D or cycloheximide. In contrast to Hep 3B cells, cells of another human hepatoma line, Hep G2, showed little induction of heme oxygenase mRNA by heat treatment. These findings suggest that heat shock treatment induces heme oxygenase mRNA in certain human hepatoma cells, but not in others.

Microsomal heme oxygenase (EC 1.14.99.3) catalyzes the rate-limiting step in the oxidative metabolism of heme which yields biliverdin (1). Heme oxygenase activity can be induced in many cell types by treatment with hemin, the substrate for the enzyme, as well as with various other non-heme substances (2). Recently it has been shown that heme oxygenase is a major 32-kDa stress protein induced in rat hepatoma cells by treatment with arsenite and cadmium (3), as well as in human fibroblasts by treatment with UVA radiation, hydrogen peroxide, and sodium arsenite (4). In rat glioma cells, heme oxygenase was also shown to be a heat shock protein (5). Similar to the rat heme oxygenase gene (5), the human gene (6) also contains a potential heat shock element (HSE) upstream from the initiation site. However, the human enzyme was reported not to be inducible in macrophages (7), glioma (7), HeLa (8), or in HL60 cells (8), or only marginally inducible in fibroblasts by heat shock treatment (4). We have recently demonstrated that heme oxygenase mRNA is inducible in human hepatoma cells in response to various stimuli including hemin and heavy metals (9). In order to determine whether heat shock also constitutes another stress leading to the induction of heme oxygenase mRNA in human hepatoma cells, we examined changes in heme oxygenase mRNA levels in two human hepatocarcinoma cell lines, Hep G2 and Hep 3B, following heat exposure.

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MATERIALS AND METHODS

Cell culture and heat treatment: Hep 3B cells were obtained from American Type Culture Collection, Rockville, MD, and Hep G2 cells were kindly provided by Dr. Barbara B. Knowles, The Wistar Institute, Philadelphia, PA. Both Hep 3B and Hep G2 cells were grown in 150 mm x 20 mm tissue-culture dishes (Corning, NY) in minimum essential medium with Earles salts supplemented with 10%(v/v) fetal-bovine serum, 100 units of penicillin/ml, 100μg of streptomycin/ml and 2 mM glutamine. Cells were seeded into culture dishes at 12.5% confluence, followed by medium replenishment after 4 days of incubation, and the heat treatment was done 24 hours after the medium replenishment. To treat cells with heat, the growth medium was removed and saved, the cells were replenished with Earles buffer solution, and incubated at various temperatures for 60 min, or at 43.5°C for various periods, as indicated in the figure legends. After heat exposure, the growth medium was added back to the cells and incubation was continued for 3 hours prior to the isolation of total RNA.

Northern blot analysis: Fifteen μg of total RNA were applied to 1.2%[w/v] agarose/formaldehyde gels (10), electrophoresed, and transferred to a sheet of Zeta-probe blotting membrane (Bio-Rad, Richmond, CA) for hybridization with appropriate probes. Levels of mRNAs were quantitated by densitometry using an LKB Ultroscan XL laser densitometer. Data were expressed as the ratio of the level in the treated cells to that in the untreated control.

cDNA probes: Probes used were human heme oxygenase cDNA (pHHO1) (6), and human HSP70 (pH2.3) (11). Each cDNA was inserted into pGEM4z vector (Promega Biotech, Madison, WI) for the transcription of an RNA probe, according to the method of Melton et al. (12).

RESULTS

Induction of heme oxygenase mRNA by heat treatment in Hep 3B cells: Fig. 1 shows the results of heat treatment on the levels of heme oxygenase mRNA in Hep 3B cells. Heat treatment for 60 min at temperatures higher than 40°C clearly

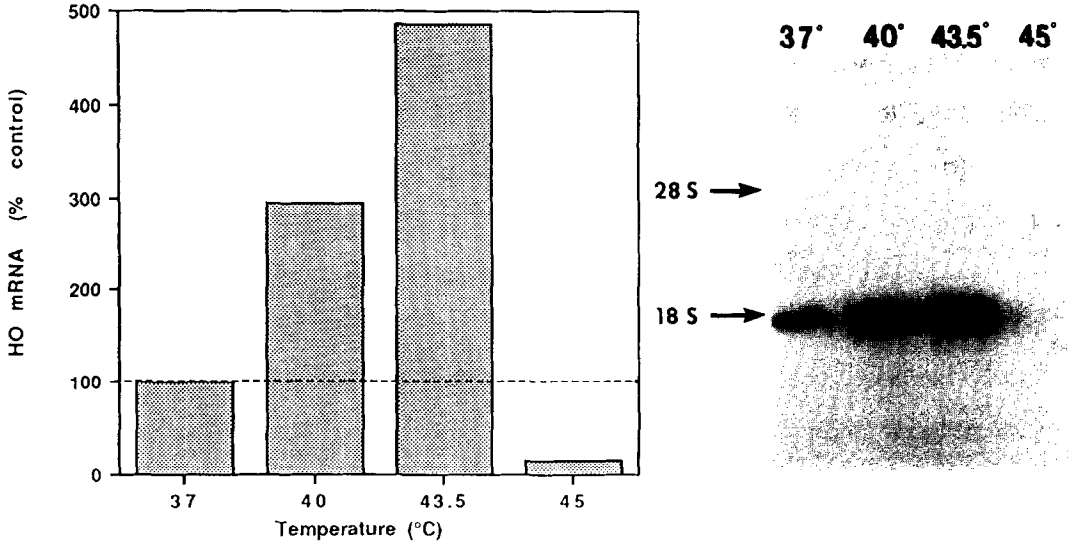

Fig. 1. Effect of heat treatment on heme oxygenase mRNA levels in Hep 3B cells. Cells were incubated for 60 min at various temperatures as indicated.

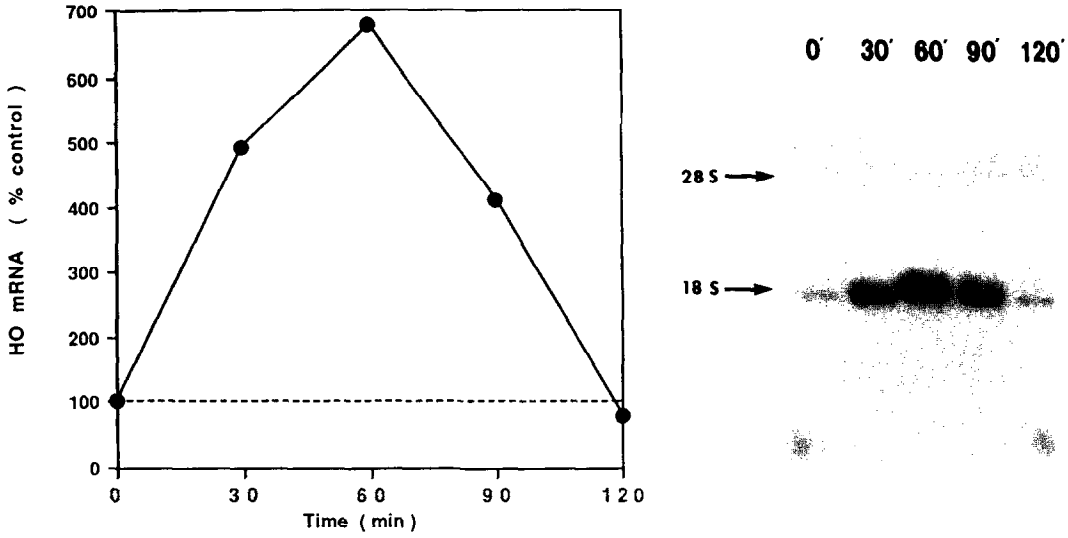

Fig. 2. Time course of heme oxygenase mRNA induction in Hep 3B cells after the commencement of heat treatment. Cells were incubated at 43.5°C.

induced heme oxygenase mRNA in Hep 3B cells. A maximal level of 5-fold induction was observed at the optimum temperature of 43.5°C. This finding was in striking contrast to that observed with Hep G2 cells which showed little induction of heme oxygenase mRNA in response to heat exposure (data not shown). Heat treatment of Hep 3B cells at 43.5°C for various time periods showed significant induction of heme oxygenase mRNA at 30 min of treatment or longer, and maximal induction was observed when cells were incubated at 43.5°C for 60 min (Fig. 2).

Blockade of the heat-mediated induction of heme oxygenase mRNA by actinomycin D and cycloheximide: When actinomycin D, a specific inhibitor of DNA-directed RNA synthesis, or cycloheximide, an inhibitor of protein synthesis, was added to Hep 3B cells at the beginning of heat treatment, the heat-mediated induction of heme oxygenase mRNA was essentially abolished (Fig. 3).

Changes in the levels of HSP70 mRNA in human hepatoma cells after heat treatment: HSP70 mRNA was induced both in Hep G2 and 3B cells after heat treatment. The maximal induction response was observed when cells were incubated at 43.5°C for 60 to 90 min. Both Hep G2 and 3B cells showed a comparable degree of induction of HSP70 mRNA (≈50-fold) (data not shown).

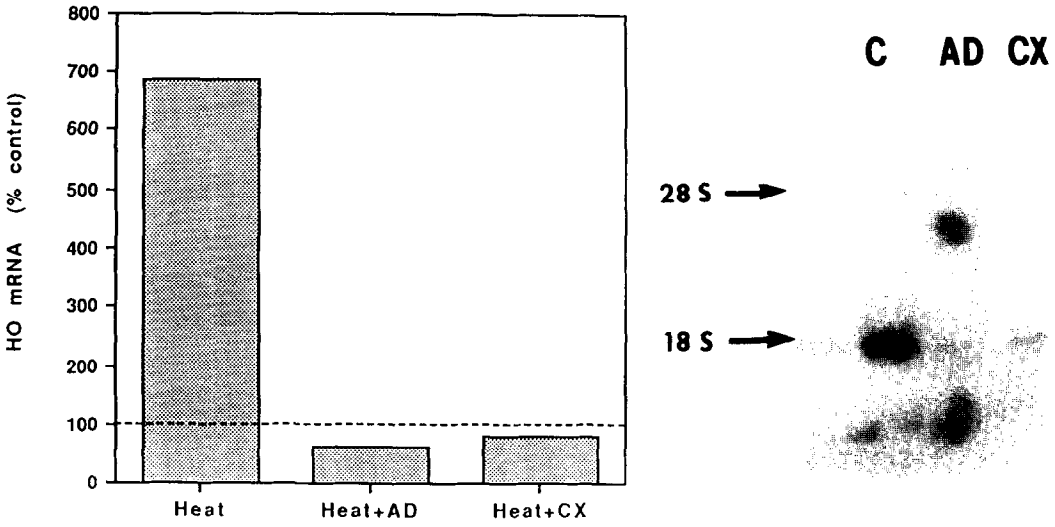

Fig. 3. Effect of actinomycin D and cycloheximide on heat-induced heme oxygenase mRNA levels in Hep 3B cells. Cells were incubated at 43.5°C for 30 min. **C**: no addition; **AD**: actinomycin D (0.25μg/ml); **CX**: cycloheximide (1μg/ml).

DISCUSSION

The results of our experiments provide evidence for the induction of heme oxygenase mRNA in human Hep 3B hepatoma cells in response to heat shock treatment. The heat-mediated induction of heme oxygenase mRNA in Hep 3B cells was temperature- and time-dependent. It also appeared to be exerted at the level of transcription, since the induction response was abolished by treatment of cells with actinomycin D. The full induction of heme oxygenase mRNA may also require the synthesis of certain protein(s), since the induction was also abolished by treatment with cycloheximide.

The heat-mediated induction of heme oxygenase mRNA in Hep 3B cells was in contrast to the findings with Hep G2 cells, which showed little induction of heme oxygenase mRNA in response to heat treatment. The refractory nature of heme oxygenase mRNA induction in Hep G2 cells by heat treatment was unique in that, in contrast, both Hep G2 and Hep 3B cells showed marked induction of heme oxygenase mRNA in response to hemin, cobalt protoporphyrin, cadmium, arsenite, and IL-6 (9). In addition, HSP70 mRNA was induced in Hep G2 cells to a comparable degree to that in Hep 3B cells after heat treatment. These findings suggest that

there is a distinct control on the gene expression of heme oxygenase and HSP70, and that the function of the HSE in the heme oxygenase gene in Hep G2 cells may be blocked, while it is not blocked in Hep 3B cells.

It has been reported that heme oxygenase mRNA was not inducible by heat treatment in several human cell lines, including U937 macrophages (7), HeLa (8), and HL60 cells (8), or that it was only marginally increased in human fibroblasts ($\leq$ 2-fold) (4). Thus it is generally assumed that human heme oxygenase is not a heat shock protein (7,8). Our findings in this study, however, clearly indicate that heme oxygenase mRNA is inducible by heat shock in certain human hepatoma cell lines such as Hep 3B, although it is little inducible in other hepatoma lines, e.g., Hep G2. There may therefore be an additional mechanism for the regulation of the HSE, which may be different depending on cell lines, in the human heme oxygenase gene.

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