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# cAMP increasing agents prevent the stimulation of heat-shock protein 70 (*HSP70*) gene expression by cadmium chloride in human myeloid cell lines

**Nuria E. Vilaboa[1,2], Consuelo Calle[2], Concepción Pérez[1,3], Elena de Blas[1], Laura García-Bermejo[1,4] and Patricio Aller[1,*]**

[1]Centro de Investigaciones Biológicas, CSIC, Velázquez 144, 28006-Madrid, Spain
[2]Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad Complutense, Madrid, Spain
[3]Instituto de Química Médica, CSIC, Madrid, Spain
[4]Departamento de Biología Celular y Genética, Universidad de Alcalá, Madrid, Spain

*Author for correspondence

## SUMMARY

**Treatment of U-937 human promonocytic cells with the cAMP increasing agents isoproterenol plus theophylline decreased the basal level of heat-shock protein 70 (*HSP70*) mRNA. In addition, the cAMP increasing agents attenuated the increase in *HSP70* mRNA and protein levels produced by cadmium chloride in U-937 and other human myeloid cell lines, reduced the capacity of cadmium treatment to generate stress-tolerance, and attenuated the cadmium-produced stimulation of heat-shock factor (HSF) binding activity. By contrast, isoproterenol plus theophylline failed to attenuate the stimulation of *HSP70* gene expression and HSF binding activity caused by heat-shock. Isoproterenol plus theophylline did not prevent the uptake of cadmium into the cells, and increased to a similar extent the intracellular cAMP levels in cadmium- and heat-treated cells. The cAMP increasing agents reduced the induction by cadmium of the *HSP27* stress gene, but failed to attenuate other cadmium-elicited stress reactions such as the inhibition of total protein synthesis. It is concluded that cAMP does not inhibit the stress response as a whole, but it interferes with some step of the pathway by which cadmium specifically stimulates HSF binding activity and as a consequence *HSP70* gene expression, in human myeloid cell lines.**

Key words: *HSP70*, cAMP, cadmium chloride, heat-shock, myeloid cell

## INTRODUCTION

A common response of all living organisms to abnormally high temperatures or other environmental or metabolic stresses is the induction of a group of highly conserved proteins, termed heat-shock proteins (HSPs) (Lindquist and Craig, 1988). The most abundant and best characterized HSPs are some proteins of approximately 70 kDa, classified as the HSP70 family. In mammals, the *HSP70* gene family includes: stress-inducible *HSP70* genes, constitutively expressed cognate *HSC70* genes, and constitutively expressed glucose-related *GRP78* (*BiP*) and *GRP75* genes (Welch, 1991). The analysis of the promoter region suggests a complex pattern of regulation for *HSP70* gene expression. In fact, in addition to the heat-shock response DNA element which confers inducibility by stress agents (HSE, which binds heat-shock transcription factors, HSFs) (Wu et al., 1986), elements which may interact with SRF-, SP1-, TFIID-, CTF-, and ATF-like factors (Wu et al., 1987; Williams et al., 1989) and with c-myc proteins (Taira et al., 1992), have been characterized in the *HSP70* promoter.

The presence of an ATF/CRE-like element might indicate that *HSP70* gene expression is regulated by cAMP. Using transient transfection assays, Choi et al. (1991) demonstrated that cAMP increasing agents stimulated the expression of a human *HSP70* promoter-driven reporter gene, and that the cAMP sensitivity was conferred by both the ATF/CRE and HSE sequences. Nonetheless, other reports indicated that cAMP increasing agents failed to stimulate the basal expression of the endogenous *HSP70* gene in intact cells (Ting et al., 1989; Murakami et al., 1991; Pizurki and Polla, 1994), although they could potentiate the stimulation produced by heat-shock (Pizurki and Polla, 1994).

In the present work we investigate the capacity of cAMP increasing agents to modulate *HSP70* gene expression at the RNA and protein levels, as well as HSF binding activity, in human myeloid leukemia cell lines. The results indicate that cAMP does not increase the basal expression of the *HSP70* gene, and it even attenuates its stimulation by cadmium chloride.

## MATERIALS AND METHODS

### Cell culture and treatments

The U-937 (Sundstrom and Nilsson, 1976), HL-60 (Collins et al., 1977) and K562 (Lozzio and Lozzio, 1975) cells used were

mycoplasma-free. The cells were grown in suspension in RPMI-1640 medium, supplemented with 10% (v/v) heat-inactivated fetal calf serum and 0.2% sodium bicarbonate and antibiotics in a humidified 5% $CO_2$ atmosphere at 37°C. Cells were seeded in 100-mm plastic dishes at the concentration of $10^5$ cells/ml and maintained in continuous logarithmic growth by passage every 2-3 days. Theophylline (Sigma Química, Madrid, Spain) and cadmium chloride (Merck, Darmstad, FRG) were dissolved in distilled water at 100 mM, while isoproterenol (Sigma) was dissolved in RPMI at 10 mM, just before application. Forskolin (Sigma) was dissolved at 10 mM in a mixture of dimethyl sulfoxide and absolute ethanol (2:1, v/v) and kept at –20°C. For treatments, the drugs were directly applied to the cultures at the desired final concentrations. For heat-shock, the cultures were transferred to an oven at 42°C for the required time-periods. For recovery after treatments, the cells were collected by centrifugation, washed once (in the case of heated cells) or three times (in the case of cadmium-treated cells) with pre-warmed (37°C) RPMI medium, and resuspended in pre-warmed drug-free culture medium. As controls, cells were subjected to the same manipulations as treated cells, in the absence of the drugs. Inhibition of cell proliferation and permeability to trypan blue were used as criteria to evaluate the toxicity of the treatments.

### Measurement of intracellular cAMP level

Aliquots of $2\times10^6$ cells were washed twice with phosphate buffered saline (PBS) without $Ca^{2+}$ and $Mg^{2+}$, resuspended in 1 ml of 6% (w/v) cold trichloroacetic acid and kept at 4°C for 1 hour. Upon centrifugation, the supernatants were extracted four times with 4 ml ethyl ether, after which the aqueous phases were dried under vacuum and finally dissolved in 1 ml of 0.05 M sodium acetate buffer, pH 5.8, containing 0.02% bovine serum albumin and 0.005% thimerosal. The cAMP content in the samples was determined with the Biotrak cAMP enzymeimmunoassay (EIA) system (dual range) (Amersham), according to the instructions described by the manufacturer.

### Measurement of intracellular cadmium level

Aliquots of $2\times10^6$ cells were washed three times with PBS without $Ca^{2+}$ and $Mg^{2+}$. Upon centrifugation, the pellets were dissolved with 1% (v/v) $HNO_3$, and the level of cadmium in the samples was measured with a Perkin-Elmer 1100 B flameless atomic absorption spectrophotometer (Norwalk, Connecticut, USA).

### Measurement of [$^3$H]valine incorporation

Untreated cells and cadmium-treated cells were washed three times with pre-warmed Hanks' solution and finally resuspended in the same solution at $5\times10^5$ cells/ml. Aliquots of 200 μl of the cell suspensions were seeded by triplicate in a 96-well plate, and 0.6 μCi of L[3,4(n)-$^3$H]valine (33 Ci/mmol) (Amersham Iberica, Madrid, Spain) was added to each of them. After incubation for 1 hour at 37°C, the cells were collected with a cell harvester and the radioactivity incorporated into proteins was measured in a 1450 MicroBeta Plus scintillation counter (Wallac Oy, Turku, Finland).

### RNA blot assays

Total cytoplasmic RNA was prepared as described previously (Aller and Baserga, 1986). RNA samples (15 μg per lane) were electrophoresed in 1.1% agarose-formaldehyde gels containing 0.1 μg/ml ethidium bromide and blotted onto nylon membranes (Hybond-N, Amersham, Buckinghamshire, UK). Ethidium bromide staining of 28 S and 18 S ribosomal RNAs was routinely checked before blotting as a control of sample loading and after blotting as a control of RNA transfer. RNA blots were prehybridized, hybridized with excess $^{32}$P-labeled cDNA probes, washed under highly stringent conditions and autoradiographed. The probes used were: the 2.3 kb human *HSP70*-specific *Bam*HI-*Hin*dIII fragment of pH2.3 plasmid (Hunt and Morimoto, 1985) (obtained through the American Type Culture Collection, catalogue no 57494), the 2.7 kb *fos*-specific *Xho*I-*Nco*I fragment of pc-fos$^{human}$ plasmid (Van Straaten et al., 1983), the 1.5-kb *Cla*I-*Eco*RI fragment of pMC413rc plasmid, which contains the third exon of human c-*myc* (Dalla Favera et al., 1982), and the entire pUCHS208 plasmid, which contains a 0.4 kb cDNA insert of human-specific *HSP27* (Stress Gene, Biotechnologies Corp., Victoria, Canada). The fragments were labeled to approximately $10^9$ cpm/μg of DNA with [α-$^{32}$P]dCTP (3,000 Ci/mmol, New England Nuclear, Boston, MA) by random hexanucleotide priming (Feinberg and Vogelstein, 1984).

### Immunoblot assays

Cells were washed with PBS and lysed in 62.5 mM Tris-HCl, pH 6.8, containing 2% SDS, 5% (v/v) β-mercaptoethanol, and 10% (v/v) glycerol. After boiling for 2 minutes at 98°C, aliquots of 10 μg protein extracts were separated on SDS-polyacrylamide (10% w/v) slab minigels (Laemmli, 1970). Electrophoretic blotting onto nitrocellulose (Hybond-C, Amersham) and immunological detection of proteins were carried out essentially as described by Towbin et al. (1979), using as the first antibody an anti-human HSP70 which specifically recognized the stress-inducible form (clone C92F34-5, Stress Gene) and as the second antibody horseradish peroxidase-conjugated rabbit anti-mouse IgG (Dakopatts, Copenhagen, Denmark). The filters were developed with an enhanced chemiluminiscence western blotting detection kit (Amersham), following the procedure described by the manufacturer.

### Gel retardation assays

Nuclear extracts from $2\times10^7$ cells were prepared according to the method of Schreibert et al. (1988) and stored at –70°C. For HSF binding assays, the partially complementary oligonucleotides 5′-GCGAAACCCCTGGAATATTCCCGACCTGGC-3′, and 5′-GGG-CCAGGTCGGGAATATTCCAGGGGTTTCG-3′ (prepared with a Gene Assembler Plus, Pharmacia LKB, Uppsala, Sweden) were used to generate a HSE-containing double strand oligoprobe, which was labeled with [α-$^{32}$P]dCTP following the method of Sambrook et al. (1989). Binding reactions were carried out for 15 minutes at room temperature in 20 μl of binding buffer (60 mM KCl, 1 mM $MgCl_2$, 12% glycerol, 1 mM 1,4-dithiothreitol, 20 mM HEPES, pH 7.9) containing 5 ng of labeled probe, 8 μg of total nuclear proteins, 1 μg of poly (dI:dC) and 1 μg salmon sperm. When required, 500 ng of unlabeled oligonucleotide was added as specific competitor. The samples were electrophoresed in 4% polyacrylamide gels, and the gels dried and autoradiographed.

## RESULTS

### *HSP70* mRNA level

Firstly, northern blot assays were carried out to investigate the effect of treatments for 1 to 6 hours with cAMP increasing agents on the basal level of *HSP70* mRNA in U-937 human promonocytic cells. It was found that the combination of 1 μM isoproterenol plus 1 mM theophylline (ISP+THP) decreased the *HSP70* mRNA level by approximately 50% (Fig. 1). Other treatments, such as dibutyryl cAMP (1 mM) or isoproterenol alone (1 μM), had null or lower effects (results not shown). Hence, the combination of ISP+THP was adopted for further experiments. ISP+THP greatly increased c-*fos* mRNA level and down regulated c-*myc* mRNA level (Fig. 1), as it is expected to occur by increasing cAMP levels (Slungaard et al., 1987; Nakamura et al., 1990).

Then, we wanted to know whether cAMP could affect the stimulation of *HSP70* gene expression caused by stress response inducers. With this aim, U-937 cells were treated for 2 and 3 hours with 25 μM cadmium chloride, either in the

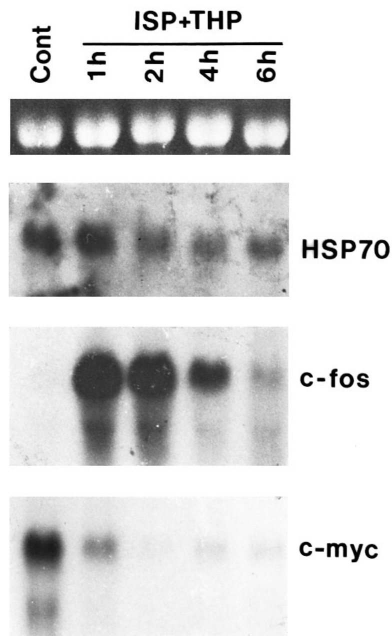


**Fig. 1.** Modulation by isoproterenol plus theophylline of the accumulation of specific mRNAs in U-937 cells. Samples of total cytoplasmic RNA extracted from either untreated cells (Cont) and cells treated for the indicated time periods with 1 µM isoproterenol plus 1 mM theophylline (ISP+THP) were used for northern blot assays. The same filter was sequentially hybridized with the indicated probes. The autoradiograph corresponding to *HSP70* was overexposed for a better detection of the basal level of this transcript. Ethidium bromide staining of 28 S ribosomal RNA in the gel is shown at the top, as a control of sample loading. The sizes of the transcripts were 2.6 kb for *HSP70*, 2.2 kb for c-*fos* and 2.3 kb for c-*myc*.

absence or the presence of ISP+THP. This concentration of cadmium, which was non toxic, was selected on the grounds of its earlier use in experiments with human monocytes (Pizurki and Polla, 1994). It was found that cadmium increased the *HSP70* mRNA level, and that the increase was greatly attenuated by presence of ISP+THP (Fig. 2A). Such an attenuation could also be observed when the cAMP increasing agent forskolin (25 µM) was used instead of ISP+THP (Fig. 2B), as well as when cadmium chloride was used at the concentrations of 100 and 150 µM (Fig. 2C), which were moderately toxic (see below). We also investigated the effect of 1 hour heating at 42°C, a moderately toxic temperature, either in the absence or in the presence of ISP+THP. This temperature was selected on the basis of its earlier use in experiments with U-937 (Twomey et al., 1993) and other human myeloid cell lines (Yufu et al., 1990; Mivechi et al., 1992). Heat-shock caused a great increase in the *HSP70* mRNA level which, by contrast with cadmium, was not attenuated and was even slightly increased by ISP+THP (Fig. 2D).

The action of ISP+THP on the cadmium- and heat-produced stimulation of *HSP70* gene expression was also studied in human HL-60 promyelocytic and K562 erythroblastoid cells. ISP+THP attenuated the increase in *HSP70* mRNA level produced by cadmium chloride, but not the increase produced by heat-shock in these cell lines (Fig. 3), as it was the case with U-937 cells.

### HSP70 protein level

*HSP70* gene expression was then measured at the protein level by means of immunoblot assays, using an antibody which specifically recognized the stress-inducible HSP70 species. The determinations were carried out in K562 cells, since HSP70 seems to be more efficiently induced in this cell line

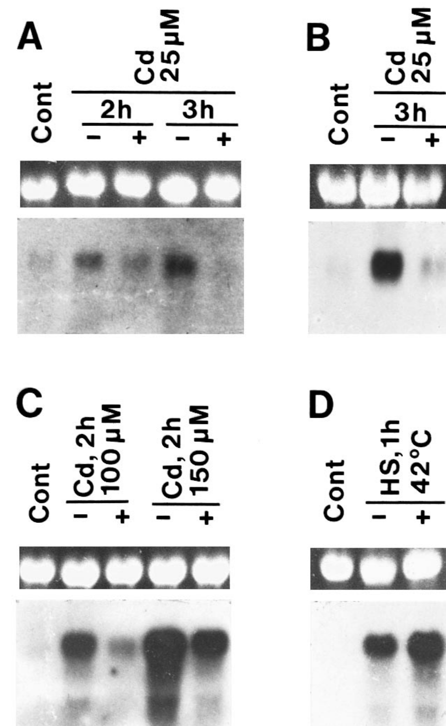


**Fig. 2.** Modulation of the *HSP70* mRNA level in U-937 cells treated with cadmium chloride (Cd) or heated (HS), either in the absence (–) or in the presence (+) of cAMP increasing agents. The used cAMP increasing agents were ISP+THP in A, C and D, and 25 µM forskolin in B. These agents were applied 10 minutes in advance of the stress treatments. All other conditions were as in Fig. 1.

than in U-937 and HL-60 cells (Mivechi, 1989; and our unpublished observations). Some of the obtained results are shown in Fig. 4. Untreated K562 cells already exhibited significant amounts of HSP70, as it was earlier reported (Mivechi, 1989). Nevertheless, the level of this protein was further increased at 3 and 6 hours of recovery after treatments with cadmium chloride and heat-shock. Such an increase was prevented when ISP+THP were applied with cadmium, but not when they were

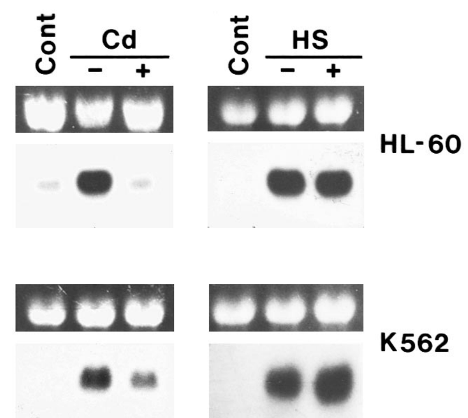


**Fig. 3.** Modulation of the *HSP70* mRNA level in HL-60 and K562 cells treated for 3 hours with 25 µM cadmium chloride (Cd) or heated for 1 hour at 42°C (HS), either in the absence (–) or in the presence (+) of ISP+THP. All other conditions were as in Figs 1 and 2.

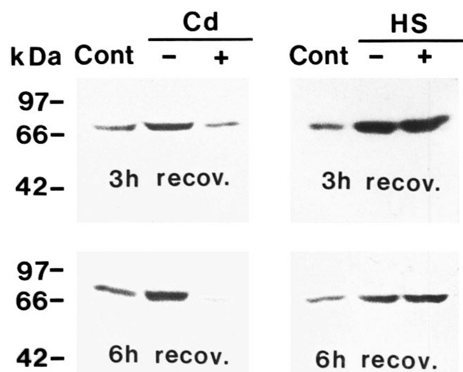


**Fig. 4.** Modulation by ISP+THP of the cadmium- and heat-produced increase in HSP70 protein level. K562 cells were treated for 3 hours with 25 µM cadmium chloride (Cd) or heated for 1 hour at 42°C (HS), either in the absence (–) or in the presence (+) of ISP+THP, and then allowed to recover for 3 or 6 hours at 37°C in the absence of the drugs. As control (Cont), unheated drug-untreated cells were used. Protein samples were assayed by immunoblot, using an antibody which specifically recognized the HSP70 stress-inducible form. Reference proteins were rabbit muscle phosphorylase *b* (97.4 kDa), bovine serum albumin (66.2 kDa) and hen egg white ovoalbumin (42.7 kDa).

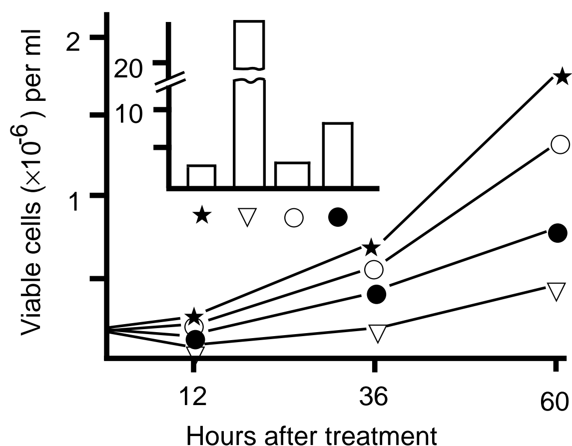


**Fig. 5.** Modulation by ISP+THP of cadmium-induced stress-tolerance. U-937 cell cultures were treated for 3 hours with 25 µM cadmium chloride either in the absence (open circles) or in the presence (filled circles) of ISP+THP, then allowed to recover for 6 hours in drug-free medium, treated again for 3 hours with 150 µM cadmium chloride alone, and finally transferred to drug-free medium. Starting at the end of the second treatment, the cell number was periodically measured. Drug-untreated cells (stars) and cells to which only the second cadmium treatment (150 µM) was applied (triangles) were subjected to the same manipulations as above, and used as controls. The inset indicates percentages of non-viable cells (cells permeable to trypan blue) at hour 12.

applied at the time of heat-shock. Hence, the results obtained at the protein level confirmed those obtained at the RNA level.

## Stress-tolerance

It is known that the stimulation of HSPs synthesis by cadmium or other stress agents makes the cells transiently resistant to ulterior stress treatments, a phenomenon known as stress-tolerance (Li et al., 1982). For this reason, we wanted to determine whether cAMP increasing agents, which attenuated *HSP70* gene induction by cadmium, could also reduce the cadmium-induced stress-tolerance. With this aim, U-937 cells were subjected to a pre-treatment with 25 µM cadmium chloride either alone or in the presence of ISP+THP, then allowed to recover in drug-free medium, and subjected again to a second treatment with 150 µM cadmium chloride alone, after which cell growth and mortality were measured at different times. Untreated cells as well as cells treated with 150 µM cadmium chloride without pre-treatment were used as controls. It was found that the growth inhibition and the mortality caused by 150 µM cadmium were attenuated when the cells were pre-treated with 25 µM cadmium alone, and that the attenuation was lower when ISP+THP were present during pre-treatment (Fig. 5). Pulse-treatments with either ISP+THP alone or 25 µM cadmium chloride alone did not significantly affect cell proliferation nor caused mortality (results not shown).

## HSF binding activity

Since the stimulation of *HSP70* gene transcription by stress treatments is mediated by the HSE (Morimoto et al., 1992), gel retardation assays were carried out to investigate whether cAMP increasing agents could affect HSF binding activity. Some of the obtained results are shown in Fig. 6. The gels revealed two types of HSF/HSE complexes which represent stress-inducible (upper band) and constitutive (lower band)

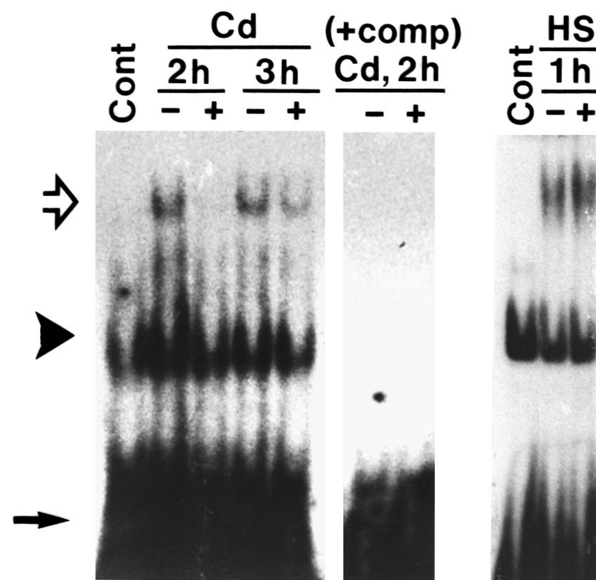


**Fig. 6.** Modulation by ISP+THP of the cadmium- and heat-induced HSF binding activity. Untreated U-937 cells (Cont), and cells treated for 2 or 3 hours with 25 µM cadmium chloride (Cd) or heated for 1 hour at 42°C (HS), either in the absence (–) or in the presence (+) of ISP+THP, were used to obtain nuclear extracts. The extracts were analyzed for HSF binding by gel retardation assays, using a double strand oligoprobe which contained the HSE. Where indicated, a 100-fold excess of unlabeled oligoprobe was used as specific competitor (+ comp). The putative position of stress-inducible oligoprobe-HSF complexes (open arrowhead), constitutive oligoprobe-HSF complexes (filled arrowhead) and free oligoprobe (arrow) are indicated at the margins.

**Table 1. Effect of ISP plus THP on cadmium uptake into U-937 cells**

| Treatment | Intracellular cadmium ($ng/10^6$ cells) |
|---|---|
| None | 0.22±0.02 |
| $CdCl_2$ 25 μM, 2 hours | 9.72±0.21 |
| $CdCl_2$ 25 μM + ISP/THP, 2 hours | 10.70±0.22 |
| $CdCl_2$ 25 μM, 3 hours | 16.89±0.77 |
| $CdCl_2$ 25 μM + ISP/THP, 3 hours | 17.47±0.77 |
| $CdCl_2$ 100 μM, 2 hours | 31.45±1.17 |
| $CdCl_2$ 100 μM + ISP/THP, 2 hours | 33.30±0.54 |
| $CdCl_2$ 150 μM, 2 hours | 31.66±0.94 |
| $CdCl_2$ 150 μM + ISP/THP, 2 hours | 31.18±0.49 |

The values represent the mean ± s.d. of at least three determinations.

HSF binding (Mosser et al., 1988). It was found that ISP+THP reduced the increase in stress-inducible HSF binding caused by cadmium, but not the increase produced by heat-shock. These results parallel those obtained at the RNA level, indicating that the cAMP-produced attenuation of *HSP70* gene expression is mediated at least in part through the HSE.

### Cadmium uptake

To determine whether the attenuation by cAMP increasing agents of the cadmium-produced stimulation of *HSP70* gene expression could be explained by a decrease in cadmium uptake into the cells, the intracellular cadmium level was measured in U-937 cells treated with different concentrations of cadmium chloride either in the absence or in the presence of ISP+THP. The results in Table 1 show that cadmium levels were similar with or without ISP+THP, indicating that the cAMP increasing agents did not affect the incorporation of this heavy metal into the cells.

### Intracellular cAMP level

Then, we wanted to determine the capacity of ISP+THP and of the stress response inducers to modulate the intracellular cAMP level in U-937 cells. The results in Table 2 indicate that cadmium chloride and heat-shock were able to increase per se the cAMP levels, and that the increase was potentiated in both cases by ISP+THP. Thus, the differential effect of ISP+THP on *HSP70* gene expression in cadmium-treated and heated cells cannot be explained by a differential modulation of the intracellular cAMP level.

### Other effects of cadmium

To determine whether cAMP could affect the induction of HSP genes other than *HSP70*, the level of *HSP27* mRNA was measured in U-937 cells treated with cadmium chloride either in the absence or in the presence of ISP+THP. The results in Fig. 7A indicate that cadmium treatment increased the amount of this RNA and that the increase was attenuated by ISP+THP, albeit to a lower level than in the case of *HSP70* mRNA.

To investigate whether cAMP could prevent stress responses other than HSP induction, the activity of total protein synthesis was determined by measuring the rate of [$^3$H]valine incorporation. It was found that cadmium chloride caused a decrease in protein synthesis activity which was not attenuated by ISP+THP (Fig. 7B). In addition, the presence of ISP+THP did not reduce the capacity of pulse-treatments with 100 and 150 μM cadmium chloride to inhibit cell growth, which is also a

**Table 2. Effect of ISP plus THP, cadmium chloride and heat-shock on the intracellular cAMP levels in U-937 cells**

| Treatment | Intracellular cAMP levels ($pmol/10^6$ cells) |
|---|---|
| None | 1.60±0.75 |
| ISP/THP, 1 hour | 16.40±4.51 |
| ISP/THP, 2 hours | 7.41±1.12 |
| ISP/THP, 3 hours | 6.25±0.75 |
| ISP/THP, 6 hours | 6.41±0.31 |
| None | 1.65±0.40 |
| $CdCl_2$ 25 μM, 2 hours | 2.84±0.76 |
| $CdCl_2$ 25 μM + ISP/THP, 2 hours | 5.62±1.89 |
| $CdCl_2$ 25 μM, 3 hours | 3.26±0.41 |
| $CdCl_2$ 25 μM + ISP/THP, 3 hours | 6.35±0.88 |
| $CdCl_2$ 150 μM, 2 hours | 2.47±0.91 |
| $CdCl_2$ 150 μM + ISP/THP, 2 hours | 3.41±0.55 |
| Heat-shock, 1 hour | 2.11±0.61 |
| Heat-shock + ISP/THP, 1 hour | 5.35±1.19 |

The values represent the mean ± s.d. of at least three determinations.

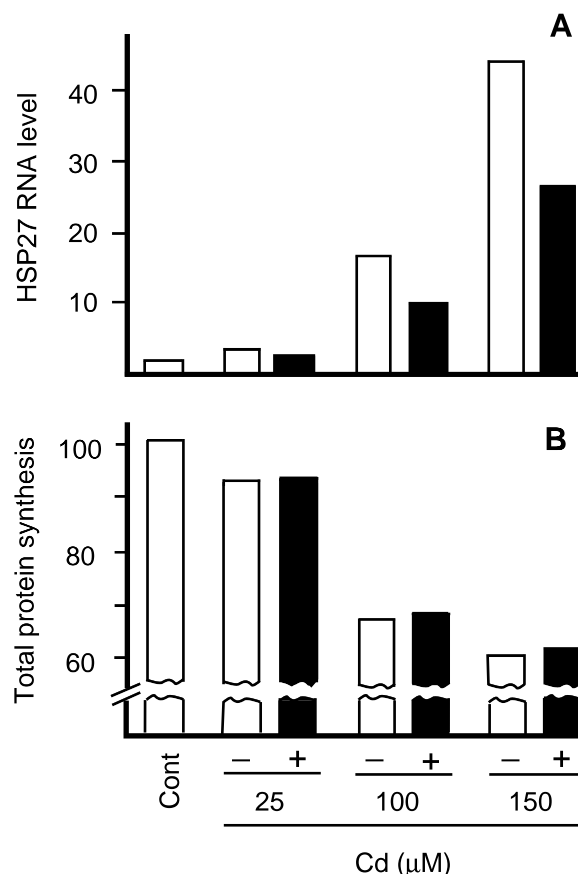


**Fig. 7.** Modulation of *HSP27* mRNA level and total protein synthesis activity in U-937 cells treated for 2 hours with the indicated concentrations of cadmium chloride, either in the absence (–) or in the presence (+) of ISP+THP. (A) The relative amounts of *HSP27* mRNA were determined by densitometry of northern blots. RNA levels in untreated cultures (Cont) were given the arbitrary value of 1. (B) At the end of the treatments the cultures were pulse-labeled for 1 hour with [$^3$H]valine, and the relative rates of radioactivity incorporated into proteins was determined. The mean incorporation in untreated cultures (Cont) was 28,520 cpm/$10^5$ cells, and was given the arbitrary value of 100.

manifestation of stress (Roti Roti et al., 1992) (results not shown).

## DISCUSSION

The results in this work indicate that cAMP increasing agents do not increase, and even slightly reduce, the basal level of *HSP70* mRNA in human myeloid leukemia cell lines. These results are in agreement with earlier observations in other human cell lines (Ting et al., 1989; Murakami et al., 1991). Thus, although cAMP elevation sufficed to induce human *HSP70* promoter activity in transfection assays (Choi et al., 1991), it appears that other signals in addition to cAMP are required to stimulate the basal expression of the endogenous *HSP70* gene.

The present results also demonstrate that cAMP increasing agents attenuate the cadmium-produced stimulation of *HSP70* gene expression in U-937 promonocytic cells and other human myeloid leukemia cell lines, measured at the RNA and protein levels. This was paralleled by the reduction of the capacity of cadmium treatment to generate stress-tolerance. Nevertheless, Pizurki and Polla (1994) reported that cAMP increasing agents did not affect the stimulation by cadmium of the synthesis of HSP70 and other HSPs in peripheral blood monocytes, measured at the protein level. The discrepancy between these and our results might reflect constitutive differences between normal circulating monocytes and cultured leukemia promonocytes. In this regard, it was earlier indicated that $H_2O_2$ stimulated HSPs synthesis in monocytes but not in U-937 cells, and that the pattern of HSPs induced by heat-shock in these cell types was not coincident (Polla et al., 1987). Whatever the case, it appears that the action of cAMP depends on the stress treatment used, since in our experiments ISP+THP failed to prevent the induction of *HSP70* by heat-shock. We are currently extending our research to the action of other *HSP70*-inducing agents.

As we have observed, ISP+THP did not interfere with the uptake of cadmium into the cells. In addition, ISP+THP failed to prevent stress reactions such as the decrease in general protein syntesis. Hence, it appears that cAMP does not inhibit the cadmium-elicited stress response as a whole, but it interferes with the specific pathway by which cadmium stimulates HSF binding activity. This was directly demonstrated by the cAMP-mediated inhibition of HSF binding observed in gel shift assays, and was furtherly corroborated by the attenuation by cAMP of the expression of the *HSP27* gene which, as *HSP70*, is regulated through the HSE. However, one cannot exclude the possibility that cAMP could additionally attenuate *HSP70* gene induction through other regulatory elements. For instance, the *HSP70* human gene promoter possesses an ATF/CRE-like element which mediated cAMP-dependent activation in transfection assays (Choi et al., 1991). Nevertheless it is known that the same cAMP-responsive elements may mediate both activation and repression of gene transcription (Lee et al., 1993; Meyer and Habener, 1993). The *HSP70* promoter also possesses a sequence which interacts with c-myc proteins (Taira et al., 1992). This must be taken into account since c-*myc* expression was greatly inhibited by ISP+THP in U-937 cells.

It has been indicated that cadmium reduces the glutathione levels, with the consequence of generating a prooxidant state in the cells (Ochi et al., 1987). In addition, several reports indicated that cAMP stimulates the removal of free radicals. For instance, isoproterenol accelerated the decay of α-tocopheroxyl radical produced in the reaction of 1,1-diphenyl-2-picrylhydrazyl with *n*-butanol (Ondrias et al., 1993). Also, dbcAMP and forskolin accelerated the transformation of ascorbate free radical into ascorbate mediated by NADH-ascorbate-free radical reductase in HL-60 cells (Rodriguez-Aguilera et al., 1993). Hence, a possible explanation for our results is that HSF is somewhat activated by the cadmium-produced free radicals, which are removed by the increase in cAMP. Experiments are in progress to investigate this hypothesis.

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## REFERENCES

**Aller, P. and Baserga, R.** (1986). Selective increase of c-*myc* mRNA levels by methylglyoxal-bis(guanylhydrazone) and novobiocin in serum-stimulated fibroblasts. *J. Cell. Physiol.* **128**, 362-366.

**Choi, H. S., Li, B., Lin, Z., Huang, L. E. and Liu, A. Y. C.** (1991). cAMP and cAMP-dependent protein kinase regulate the human heat shock protein 70 gene promoter activity. *J. Biol. Chem.* **266**, 11858-11865.

**Collins, S., Gallo, R. and Gallagher, R.** (1977). Continuous growth and differentiation of human myeloid leukemic cells in suspension culture. *Nature* **270**, 347-349.

**Dalla Favera, R., Gelmann, E. P., Martinotti, S., Franchini, G., Papas, T., Gallo, R. C. and Wong-Staal, F.** (1982). Cloning and characterization of different human sequences related to the onc gene (v-*myc*) of avian myelocytomatosis virus (MC29) *Proc. Nat. Acad. Sci. USA* **79**, 6497-6501.

**Feinberg, A. P. and Vogelstein, B.** (1984). A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **137**, 266-267.

**Hunt, C. and Morimoto, R. I.** (1985). Conserved features of eukaryotic hsp70 genes revealed by comparison with the nucleotide sequence of human hsp70. *Proc. Nat. Acad. Sci. USA* **82**, 6455-6459.

**Laemmli, U. K.** (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.

**Lee, K. A. W. and Masson, N.** (1993). Transcriptional regulation by CREB and its relatives. *Biochim. Biophys. Acta* **1174**, 221-233.

**Li, G. C., Shrieve, D. C. and Werb, Z.** (1982). Correlations between synthesis of heat-shock proteins and development of tolerance to heat and to adryamicin in Chinese hamster fibroblasts: heat shock and other inducers. In *Heat-Shock: From Bacteria to Man* (ed. M. J. Schelesinger, M. Ashburner and A. Tissières), pp. 395-404. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

**Lindquist, S. and Craig, E. A.** (1988). The heat-shock proteins. *Annu. Rev. Genet.* **22**, 631-677.

**Lozzio, C. B. and Lozzio, B. B.** (1975). Human chronic myelogenous leukemia cell line with positive Philadelphia chromosome. *Blood* **45**, 321-324.

**Meyer, T. E. and Habener, J. F.** (1993). Cyclic adenosine 3′,5′-monophosphate response element binding protein (CREB) and related transcription-activating deoxyribonucleic acid binding proteins. *Endocrinol. Rev.* **14**, 269-290.

**Mivechi, N. F.** (1989). Heat sensitivity, thermotolerance, and profile of heat shock protein synthesis of human myelogenous leukemias. *Cancer Res.* **49**, 1954-1958.

**Mivechi, N. F., Monson, J. M. and Hahn, G. M.** (1992). Lower heat shock factor activation and binding and faster rate of HSP-70A messenger RNA turnover in heat sensitive human leukemias. *Cancer Res.* **52**, 6815-6822.

**Morimoto, R. I., Sarge, K. D. and Abravaya, K.** (1992). Transcriptional regulation of heat shock genes. A paradigm for inducible genomic responses. *J. Biol. Chem.* **267**, 21987-21990.

**Mosser, D. D., Theodorakis, M. G. and Morimoto, R. I.** (1988). Coordinate changes in heat shock element-binding activity and HSP70 gene transcription rates in human cells. *Mol. Cell. Biol.* **8**, 4736-4744.

**Murakami, T., Ohmori, H., Gotoh, S., Tsuda, T., Ohya, R., Akiya, S. and Higashi, K.** (1991). Down modulation of N-myc, heat-shock protein 70, and nucleolin during the differentiation of human neuroblastoma cells. *J. Biochem.* **110**, 146-150.

**Nakamura, T., Datta, R., Sherman, M. L. and Kufe, D.** (1990). Regulation of c-*jun* gene expression by cAMP in HL-60 myeloid leukemia cells. *J. Biol. Chem.* **265**, 22011-22015.

**Ochi, T., Takahashi, K. and Ohsawa, M.** (1987). Indirect evidence for the induction of a prooxidant state by cadmium chloride in cultured mammalian cells and a possible mechanism for the induction *Mutat. Res.* **180**, 257-266.

**Ondrias, K., Misik, V., Brezova, V. and Stasko, A.** (1993). Reaction kinetics of alpha-tocopheroxyl radical with biologically and pharmacologically active substances *Free Radical Res. Commun.* **19**, 17-28.

**Pizurki, L. and Polla, B. S.** (1994). cAMP modulates stress protein synthesis in human monocytes-macrophages. *J. Cell. Physiol.* **161**, 169-177.

**Polla, B. S., Healy, A. M., Wojno, W. C. and Krane, S. M.** (1987). Hormone 1$\alpha$,25-dihydroxyvitamin $D_3$ modulates heat shock response in monocytes. *Am. J. Physiol.* **252**, C640-C649.

**Rodriguez-Aguilera, J. C., Navarro, F., Arroyo, A., Villalba, J. M. and Navas, P.** (1993). Transplasma membrane redox system of HL-60 cells is controlled by cAMP *J. Biol. Chem.* **35**, 26346-26349.

**Roti Roti, J. L., Mackey, M. A. and Higashikubo, R.** (1992). The effects of heat shock on cell proliferation. *Cell Prolif.* **25**, 89-99.

**Sambrook, J., Fritsch, E. F. and Maniatis, T.** (1989). *Molecular cloning: A Laboratory Manual,* 2nd edn. 11.40-11.44. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.

**Schreibert, E., Matthias, P., Müller, M. M. and Schaffner, W.** (1988). Identification of a novel lymphoid specific octamer binding protein (OTF-2B) by proteolytic clipping bandshift assay (PCBA). *EMBO J.* **7**, 4221-4229.

**Slungaard, A., Confer, D. L. and Schubach, W. H.** (1987). Rapid transcriptional down-regulation of c-*myc* expression during cyclic adenosine monophosphate-promoted differentiation of leukemic cells. *J. Clin. Invest.* **79**, 1542-1547.

**Sundstrom, C. and Nilsson, K.** (1976). Establishment and characterization of a human hystiocytic lymphoma cell line (U-937). *Int. J. Cancer* **17**, 565-577.

**Taira, T., Negishi, Y., Kihara, F., Iguchi-Ariga, S. M. M. and Ariga, H.** (1992). c-*myc* protein complex binds to two sites in human hsp70 promoter region. *Biochim. Biophys. Acta* **1130**, 166-174.

**Ting, L. P., Tu, C. L. and Chou, C. K.** (1989). Insulin-induced expression of human heat-shock protein gene hsp70. *J. Biol. Chem.* **264**, 3404-3408.

**Towbin, H., Stachlin, T. and Gordon, J.** (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Nat. Acad. Sci. USA* **76**, 774-781.

**Twomey, B. M., McCallum, S., Isenberg, D. A. and Latchman, D. S.** (1993). Elevation of heat-shock protein synthesis and hsp gene transcription during monocyte to macrophage differentiation of U937 cells. *Clin. Exp. Immunol.* **93**, 178-183.

**Van Straaten, F., Müller, R., Curran, T., Van Beveren, C. and Verma, I. M.** (1983). Complete nucleotide sequence of a human c-*onc* gene: deduced amino acid sequence of the human c-*fos* protein. *Proc. Nat. Acad. Sci. USA* **80**, 3183-3187.

**Welch, W. J.** (1991). The role of heat-shock protein as molecular chaperones. *Curr. Opin. Cell Biol.* **3**, 1033-1038.

**Williams, G. T., McClanahan, T. L. and Morimoto, R. I.** (1989). E1a transactivation of the human HSP70 promoter is mediated through the basal transcriptional complex. *Mol. Cell Biol.* **9**, 2574-2587.

**Wu, B. J., Kingston, R. E. and Morimoto, R. I.** (1986). Human HSP70 promoter contains at least two distinct regulatory domains. *Proc. Nat. Acad. Sci. USA* **83**, 629-633.

**Wu, B. J., Kingston, R. E. and Morimoto, R. I.** (1987). Detection of three protein binding sites in the serum-regulated promoter of the human gene encoding the 70-kDa heat shock protein. *Proc. Nat. Acad. Sci. USA* **84**, 2203-2207.

**Yufu, Y., Nishimura, J., Ideguchi, H. and Nawata, H.** (1990). Enhanced synthesis of heat-shock proteins and augmented thermotolerance after induction of differentiation in HL-60 human leukemia cells. *FEBS Lett.* **268**, 173-176.

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