EPINEPHRINE INDUCES 72-kD HEAT SHOCK PROTEIN (HSP72) IN CULTURED HUMAN FIBROBLASTS

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Abstract: In order to know the stress response of cells to epinephrine, we examined the induction of 72-kD protein (HSP 72) in cultured human fibroblasts using an immunofluorescence method. HSP 72 was induced strongly and rapidly after epinephrine treatment at a final concentration of 2.7×10^{-5} M which corresponds to the concentration of clinical use. When propranolol (1×10^{-4} M) was added to a culture medium containing epinephrine, the onset and peak of HSP 72 induction of fibroblasts were delayed compared to those observed after epinephrine treatment alone.

These results suggest that epinephrine is actually a potent inducer of HSP 72 and that the cells are stressed by epinephrine at a concentration of routine use in surgery. Furthermore, the induction of HSP 72 by epinephrine is considered to be related to its β-adrenergic action.

Index Terms

stress protein, HSP 72, epinephrine, fibroblast

INTRODUCTION

Epinephrine is a useful drug because it prevents bleeding at operation sites and inhibits the washout of local anesthetics from the injection sites. However, epinephrine has deleterious effect directly or indirectly on the cells. Injection of epinephrine might cause local tissue necrosis and decrease skin flap survival[1,2,3]. It has been reported that these toxicities are caused not only by the indirect action of epinephrine, i. e., reduction of local blood flow by vasoconstriction, but also by direct action[4]. There are many reports about the indirect action of epinephrine[1,2,3,5,6]. However, there are only few reports about the direct toxic action of epinephrine, and the details of this action's mechanism are not certain.

When cells are exposed to environmental stresses, they respond by synthesizing a characteristic group of proteins called heat shock proteins (HSPs) or stress proteins[7,8]. Of the various HSPs, 72-kD protein (HSP 72) is highly stress inducible. After exposure of cells to stress, HSP 72 accumulates in the nucleus and more prominently in the nucleoli[7]. One of the proposed roles of HSP 72 is to limit or repair the damage to cells caused by various stressful conditions such as UV irradiation, exposure to chemical agents or oxidants, infection, ischemic condition, trauma, and psychological stress[9,10,11,12,13,14]. It is well known that HSP 72 is a useful marker for estimating the stress response of the cells to various environmental factors. Induction of HSP 72 has been studied in various fields including plastic surgery[15,16].

In the present study, to know the stress response of the cells to epinephrine, we examined the

HSP 72 induction in cultured normal human fibroblasts.

MATERIALS AND METHODS

Cell line and medium

W 138 VA 13 cell line (SV 40-transformed, normal human fibroblasts) was used. The cells were cultured in a humidified incubator containing 5 % CO_2 in air. The culture medium was Eagle's minimum essential medium supplemented with 10 % new born calf serum (Nacalai Tesque, Kyoto, Japan).

Chemicals

The chemicals used in this study were epinephrine (Bosmin, 1 mg/1 ml, Daiichi Pharmacy, Tokyo, Japan), propranolol (Inderal, 2 mg/2 ml, Zeneca Pharmaceuticals, Cheshire, U. K.), and phentolamine (Regitine, 10 mg/1 ml, Ciba, Basel, Switzerland). These chemicals were diluted with culture medium, and final concentrations of epinephrine, propranolol, and phentolamine in the culture medium were 2.7×10^{-5} M (equivalent to 1 : 200000), 1×10^{-4} M, and 1×10^{-4} M, respectively.

Chemical treatments

An appropriate number of cells grown on the glass coverslips were transferred to 100-mm

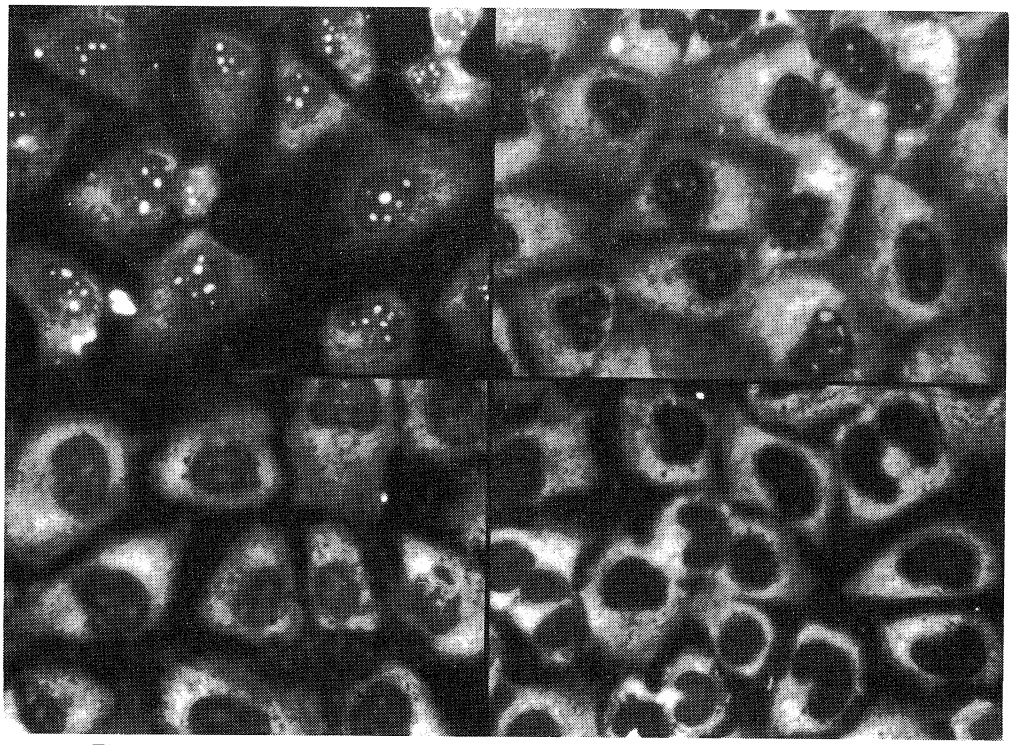

Fig. 1. The HSP72 induction was evaluated by the intensity of nuclear immunofluorescence which was graded as follows ; +++ : strongly positive (*above left*), ++ : positive (*above right*), + : weakly positive (*below left*), − : negative (*below right*). (magnification ×400)

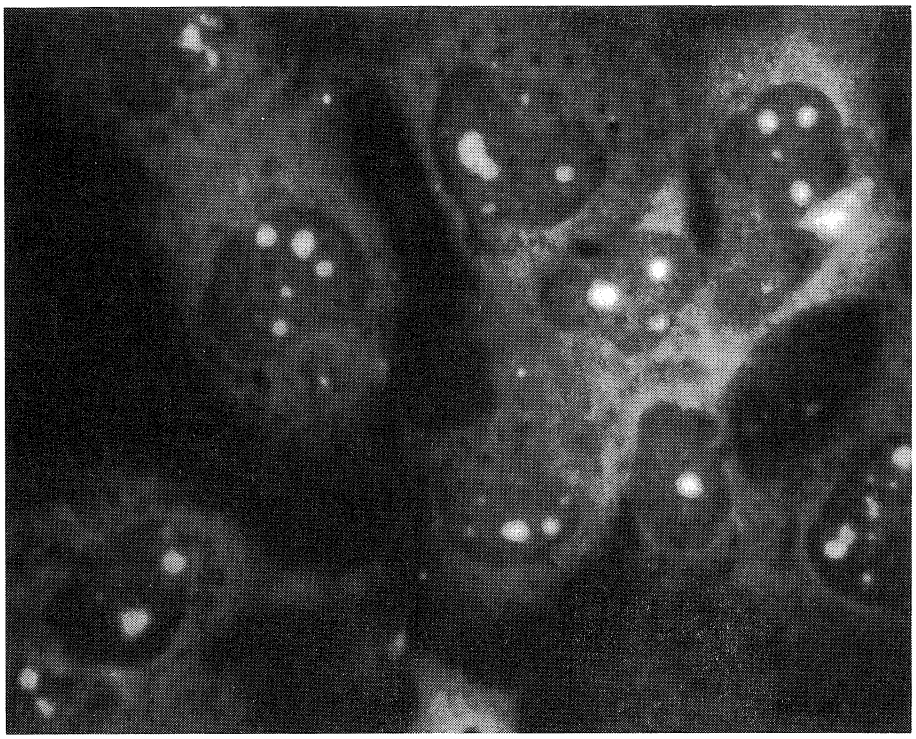

Fig. 2. The cells were incubated at 45℃ for 1hr and then further incubated at 37℃ for 4hr. The grade corresponds to strongly positive. (magnification ×400)

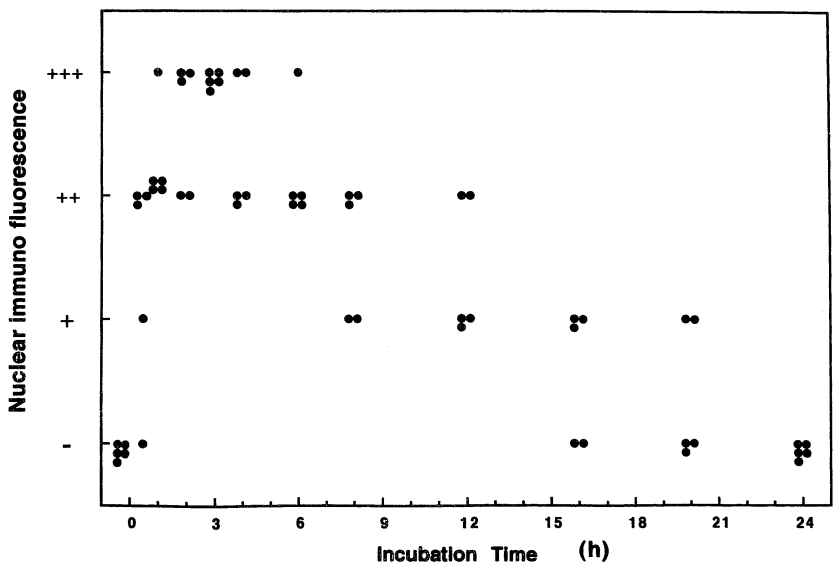

Fig. 3. The time course of HSP72 induction during epinephrine treatment.

Falcon plastic dishes containing medium with chemicals.

Immediately after treatment with epinephrine for 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 20, and 24 hr, or treatment with propranolol, phentolamine, epinephrine+propranolol, and epinephrine+phentolamine for 1, 3, 6, 9, 12, 16, 20, 24 hr, the cells grown on the glass coverslips were washed with phosphate-buffered saline (PBS). Then, the glass coverslips were fixed with 100 % methanol at 4℃.

Moreover, after treatment with epinephrine for 0.5, 1, 3, and 12 hr, glass coverslips were further incubated in medium only at 37℃ for various periods in a 5 % CO_2 incubator. The cells were then washed with phosphate-buffered saline (PBS) and fixed with 100 % methanol at 4 ℃.

Heat treatment

The analysis of HSP 72 induction after heat treatment was performed as positive control (12).

The cells grown on the glass coverslips were transferred to a culture dish containing medium

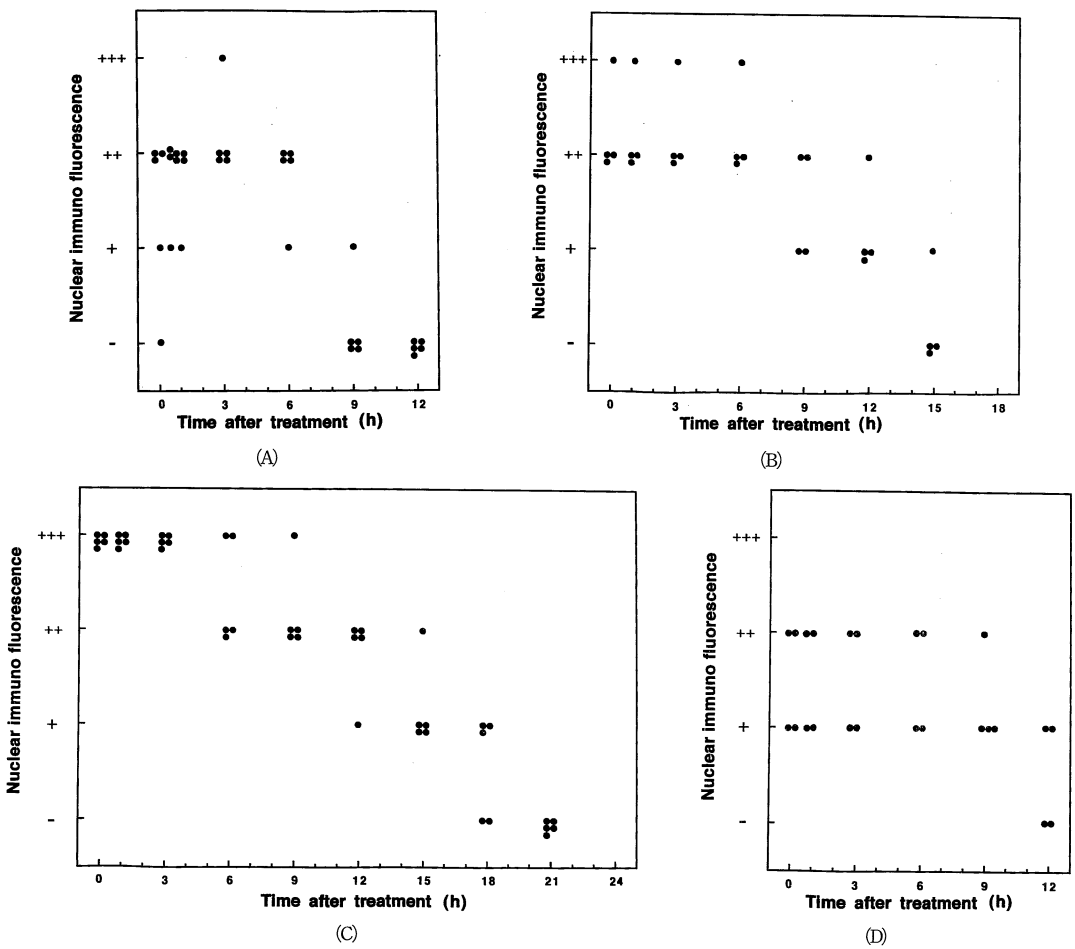

Fig. 4. The time course of HSP72 induction after various periods of epinephrine treatment; After epinephrine treatment for 0.5hr (A), 1hr (B), 3hr (C), and 12hr (D).

previously kept at 45℃, and incubated at 45℃ for 1 hr in an incubator containing 100 % air. After heat treatment, the glass coverslips were transferred to a culture dish containing medium previously kept at 37℃, and further incubated at 37℃ for 4 hr in a 5 % CO_2 incubator. Then the glass coverslips were washed with PBS and fixed with 100 % methanol at 4℃.

Indirect immunofluorescence

Analysis of the intracellular distribution of the 72-kD protein was carried out by the immunofluorescence method using a mouse monoclonal antibody (Amersham International PLC, Amersham, U. K.) specific for the 72-kD heat shock protein. The cells were incubated with the

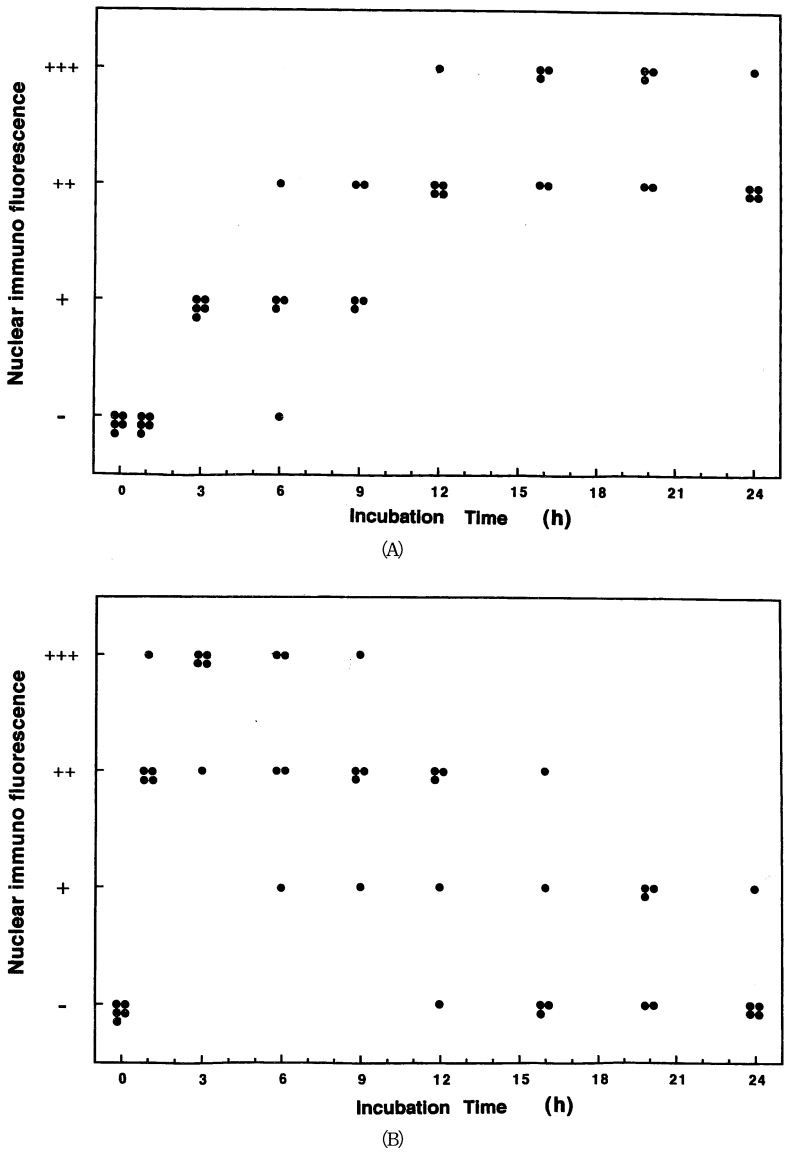

Fig. 5. The time course of HSP72 induction during treatment with epinephrine + propranolol (A) and epinephrine + phentolamine (B).

mouse monoclonal anti-HSP 72 antibody (diluted 1 : 80 in PBS) at 37℃ for 40 min followed by extensive washing, and then incubated with fluorescein isothiocyanate-conjugated horse anti-mouse IgG antibody (Vector Laboratories, Inc., Burlingame, CA) at 37℃ for 40 min. After a final washing with PBS, the glass coverslips were mounted and the cells were photographed with a fluorescence microscope (Olympus, Tokyo).

Colony froming ability after treatments with chemicals

The cells were inoculated into 100-mm Falcon plastic dishes at a density of 3×10^3 cells per dish, and incubated at 37℃ for 20 hr. After chemical treatments for various periods up to 24

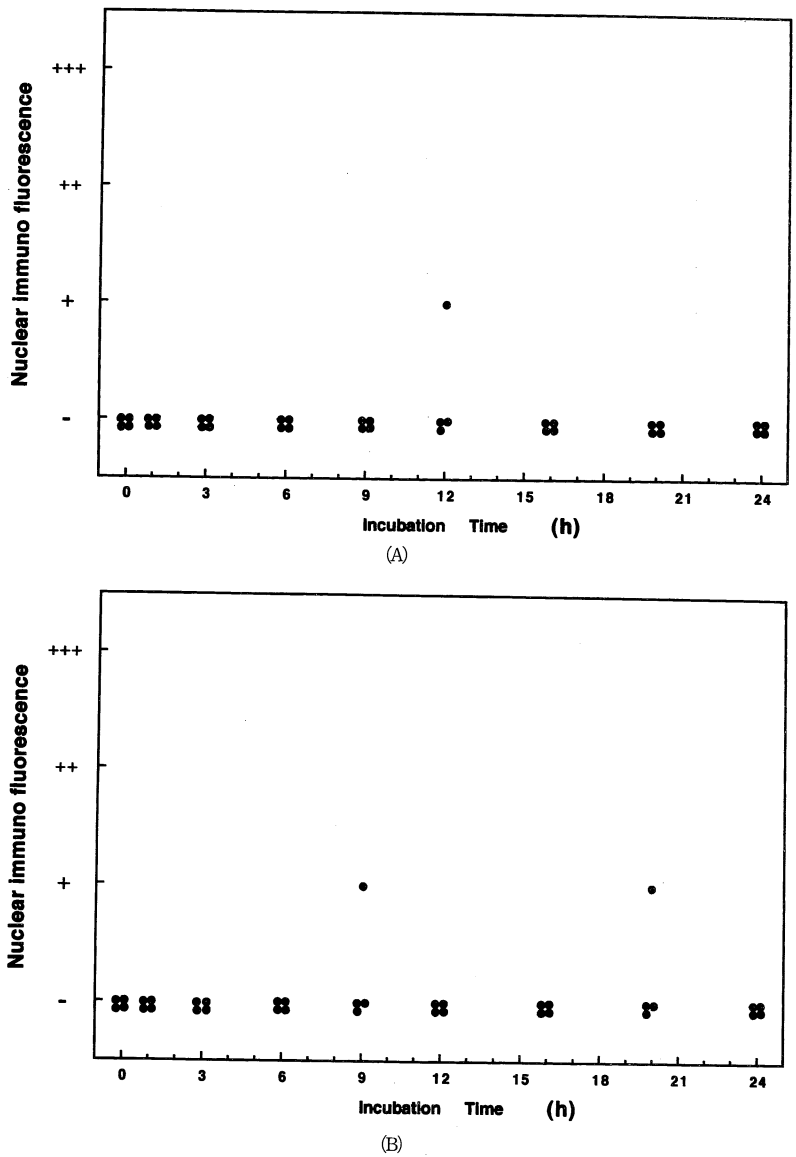

Fig. 6. The time course of HSP72 induction during treatment with propranolol (A) and phentolamine (B).

hr, the culture medium was changed to a fresh medium and the cells were further incubated at 37℃ for 14 days in a 5 % CO_2 incubator. Then, the cells were washed with PBS, fixed with methanol, and stained with Giemsa. Macroscopic colonies with more than 30 cells per colony were counted visually and the relative survival was calculated.

RESULTS

Analysis of HSP 72 induction

The HSP 72 induction was evaluated by the intensity of nuclear immunofluorescence (Fig. 1). Analysis of HSP 72 induction was performed for four or five glass coverslips in each treatment.

A) Heat treatment :

After heat treatment, strong nuclear immunofluorescence was detected (Fig. 2).

B) Chemical treatments :

The nuclear immunofluorescence was first detected at 0.5 hr after epinephrine treatment. The intensity of nuclear immunofluorescence increased until 3 hr treatment with epinephrine. After 4 hr treatment with epinephrine, nuclear immunofluorescence decreased gradually and disappeared at 24 hr treatment of epinephrine (Fig. 3). After epinephrine treatment for 0.5, 1, 3, or 12 hr, when the cells were further incubated in an epinephrine free medium, the intensity

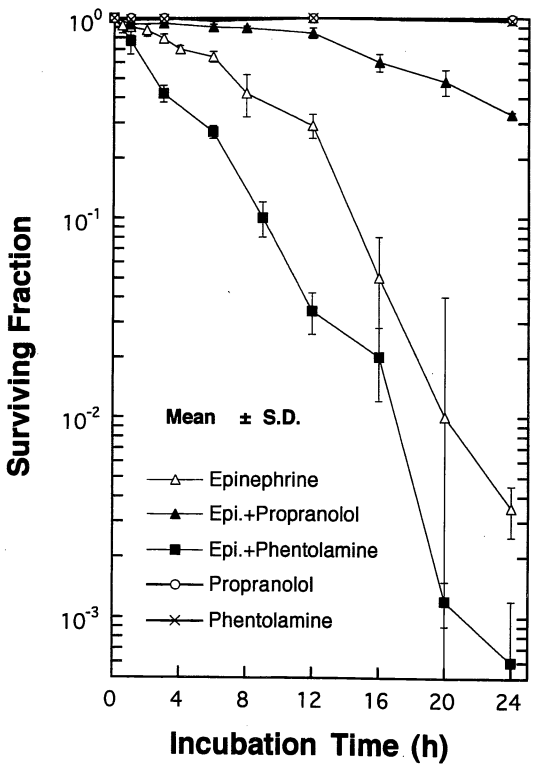

Fig. 7. Cell survival rate of various chemical treatments.
Point represents mean value of five experiments.

of the nuclear immunofluorescence decreased gradually (Fig. 4-A, -B, -C, -D). When propranolol was added to a culture medium containing epinephrine, the onset and the peak of nuclear immunofluorescence were delayed compared to those observed after treatment with epinephrine alone (Fig. 5-A). In contrast, when phentolamine was added to a culture medium containing epinephrine, immunofluorescence patterns were similar to those observed after treatment with epinephrine alone (Fig. 5-B). The nucear immunofluorescence was not observed after treatment with propranolol or phentolamine for up to 24 hr(Fig. 6-A, -B).

Cell curvival rate (Fig. 7)

The cell survival rate decreased gradually in a treatment time-dependent manner. When propranolol was added to a culture medium containing epinephrine, the cell survival rate was markedly increased. When phentolamine was used instead of propranolo, the cell survival rate did not increase but decreased more than that of epinephrine treatment alone. The cell survival rate did not change by the addition of either propranolol or phentolamine alone.

DISCUSSION

In the present study, we have demonstrated that in cultured human fibroblasts, HSP 72 was induced by epinephrine. The induction of HSP 72 was observed at 30 min treatment with epinephrine. At 3 hr treatment with epinephrine, the HSP 72 induction has reached a maximum level. The magnitude of HSP 72 induction by epinephrine was as strong as that by heat treatment. These findings suggest that epinephrine can be considered as a strong inducer of HSPs at a concentration of routine use in surgery. Moreover, when the cells were further incubated in an epinephrine free medium, the magnitude of HSP 72 showed no increase compared with that observed immediately after epinephrine treatment during the time course. These findings indicate that the time lag between initiation of epinephrine exposure and HSP 72 induction is shorter than that of other inducers of HSP 72 such as heat or ultraviolet-C irradiation[12], and that HSP 72 is induced rapidly by epinephrine. The induction of HSP 72 increased until 3 hr treatment with epinephrine, and thereafter decreased gradually. On the other hand, the cell survival rate decreased gradually in a treatment time-dependent manner. Especially, after 12 hr treatment with epinephrine, the magnitude of HSP 72 induction and cell survival rate were markedly decreased. These findings suggest that the cells were damaged to such an extent that they could not synthesize HSP 72, and it may be speculated that decreased synthesis of HSP 72 accelerates cell damage.

Induction of HSP 72 by epinephrine was attenuated by propranolol(β-antagonist), but not by phentolamine (α-antagonist). In addition, the decrease in the survival rate by epinephrine treatment was improved by the addition of propranolol. These results indicate that epinephrine -induced stress response in cultured human fibroblasts might be mediated by β-adrenergic action of epinephrine.

Epinephrine activates adenylate cyclase and accumulates cyclic-AMP[17,18], which operates as a second messenger and leads to various physiological effects on cells[18]. The relationship between β-receptor stimulation and cell death remains uncertain. However, it is considered that β-adrenergic action of epinephrine causes an increase in cellular metabolism and accumulation of metabolic byproducts, and leads to cell death[4]. HSP 72 might be induced by responding to abnormal proteins produced by the toxic action of epineprrine, and induced HSP 72 could

play an important role in protecting cells from abnormal proteins[19]. It has been reported that cyclic-AMP levels showed marked and rapid increase after epinephrine treatment in SV-40 transformed VA 13 cells[20]. A strong increase of cyclic AMP might cause drastic changes to cells, and induce HSP 72 strongly and quickly.

When phentolamine was added to a culture medium containing epinephrine, the cell survival rate decreased. The α-adrenergic action has been shown to inhibit the β-adrenergic action[17,18]. Phentolamine, α-antagonist, inhibits α-adrenergic action. Therefore, β-adrenergic action is relatively stimulated. This may be the reason for the decrease in the cell survival rate by the addition of phentolamine. However, the time course of HSP 72 induction during treatment was similar to that observed after treatment with epinephrine alone. These results indicate that the magnitude of HSP 72 induction is not necessarily dependent on the intensity of β-adrenergic stimulation.

Recently, it has been reported that cellular ATP, or the metabolic consequences associated with ATP depletion, may be threshold factors for HSP 70 expression[21]. In epinephrine-treated cells, there may be also some threshold factors for HSP 72 induction.

It has been proposed that induction of HSP 72 plays an important role in the development of resistance to stressful conditions such as heat[22] and ischemia[23,24]. In plastic surgery, improvement of skin-flap survival[16] and reduction of skeletal muscle injury[15] had been discussed in the relationship with HSP 70 induction after heat shock treatment. A new method may be developed for protecting cells from toxic conditions by using the protective role of HSP 72[25].

Our finding, that HSP 72 was strongly induced by epinephrine treatment, may suggest that cells or tissues can acquire resistance to various toxic conditions by "pre-treatment with epinephrine". In clinical practice, it may be possible that if organs or tissues are pre-treated with epinephrine for appropriate periods and at an appropriate concentration, they can be protected from toxic factors such as ischemia, exposure to chemicals, infection, trauma, and irradiation. In plastic surgery, epinephrine might protect skin from the necrosis caused by subsequent severe ischemic injury, although it is well known that epinephrine causes ischemic condition and leads to skin necrosis. The clinical significance of the induction of HSP 72 by epinephrine remains to be elucidated.

In conclusion, we demonstrated that epinephrine induces HSP 72 strongly and rapidly in WI 38 VA 13 cells, and that the cells were actually stressed by epinephrine at a concentration of routine use in surgery. Furthermore, it was revealed that the induction of HSP 72 was closely related to β-adrenergic action of epinephrine.

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