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Effects of Hyperthermia on the Myoblast Differentiation and Protein Synthesis *in vitro**

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培養한 筋原細胞의 分化와 蛋白質 合成에 미치는 溫熱處理의 効果

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요 약

온열처리가 근세포의 분화에 어떤 영향을 미치는 지를 알아보기 위하여 배양한 근세포에 여러가지 온열처리를 한 다음, 단백질 합성, 세포증식, 융합지수, creatine kinase(CK)의 활성 및 cholesterol 함량의 변화를 조사하였다.

배양한지 24시간지나 45°C에서 1 hr의 온열처리를 하면 근세포의 융합과 CK 활성이 지연되었으며, 55시간지나 같은 온열처리를 하면 세포막내(세포내 양의 95% 이상)의 cholesterol 양이 일시적으로 증가함과 아울러 세포융합이 지연되었다. 그러나 세포증식은 대조군과 뚜렷한 차이가 없었다. 이상과 같은 실험 결과로부터 온열처리가 분화에 미치는 영향은 온열처리를 받게되는 근원세포의 분화정도에 따라 차이가 있으며 온열처리에 따른 cholesterol 양의 일시적인 증가가 근세포 융합에 영향을 미칠 수 있다는 가능성이 제시되었다.

한편, 근세포는 온열처리를 받으면 평소의 단백질 합성 수준이 떨어짐과 더불어 heat shock protein(HSP)합성이 증대 내지는 유도 되었으며 HSP 합성의 유도는 actinomycin D의 처리로 억제되었다. 또한 온열처리로 근세포는 열내성(thermotolerance)을 얻어 세포융합과 CK 활성은 동일한 온열처리를 4시간 간격으로 두번 주어도 한번 주었을 경우와 차이가 없었으며 두번째 온열처리에 의해서는 새로운 HSP 합성이 유도되지도 않았다.

INTRODUCTION

The term 'cell differentiation' has been used to describe structural and functional modi-

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fications of an unspecialized cell into a specialized one. The mechanism of cell differentiation is one of the urgent questions to be answered in life science.

Myoblasts, derived from a vertebrate embryo, are a very useful model system to study the mechanism of cell differentiation, because embryonic myogenic cells are cultivated easily *in vitro* and they show a variety of biochemical as well as morphological characteristics. In the beginning, the myoblasts proliferate and are bipolar, and then recognize one another. Thereafter, they become postmitotic multinucleated myotubes through a rapid fusion of plasma membrane(Konigsberg, 1963; Bischoff and Holtzer, 1969; O'Neill and Stockdale, 1972; Bonner *et al.*, 1982). Coincidently, they abruptly start to synthesize muscle-specific proteins, such as creatine kinase(Turner *et al.*, 1974; Caravatti *et al.*, 1979), aldolase(Turner *et al.*, 1974), myosin(Vertel and Fischman, 1975), and acetylcholine receptors(Paterson and Prives, 1973).

Bischoff and Holtzer (1969) suggested that myogenic cells have their own intrinsic programs to differentiate, but other studies proved that environmental changes, such as frequent changes of medium or decreasing the cell density had influence on the differentiation of myoblasts(Konigsberg, 1971; Slater, 1976; Yeoh and Holtzer, 1977). Linkhart *et al.* (1981) proposed that the depletion of mitogenic factor in the medium lead to a large protraction of G_1 stage of the cell cycle and to the cell fusion. In addition, some studies suggested that cells release fusion-promoting factors into the medium(Doering and Fishman, 1977; Ha, *et al.* 1984). However, the mechanism of myoblast differentiation *in vivo* or *in vitro* has not been worked out.

In the long run, the cell differentiation is the result of regulation of gene expression. In ***Drosophila***, hyperthermia induced new pupping patterns of the polytene chromosomes in sailvary glands(Ashburner and Bonner, 1979). When stressed by heat shock, cells of most organisms respond with the increase and/or induction of specific protein synthesis, the so-called heat shock proteins(HSPs) and the reduction in the normal protein synthesis. At the same time, the cells exposed to a nonlethal heat shock acquire a transient resistance to subsequent exposures to elevated temperatures(Henle et al., 1978; Li and Hahn, 1980). This phenomenon has been termed as thermotolerance(Henle and Dethlefsen, 1978). Thus, heat-treated cells undergo a change in the pattern of gene expression and acquire thermal resistance.

Therefore, it is of interest to investigate effects on cultured myoblasts of hyperthermia which induces the new pattern of gene expression. A number of laboratories have showed that the exposure of myogenic cells to a temperature higher than their normal growth temperature resulted in the production of a special set of heat shock proteins and that myogenic cells slowly recovered normal protein synthesis when these cells were allowed to recover from heat treatment at normal growth temperature (Atkinson *et al.*, 1981; Kim *et al.*, 1983; Kim and Shuman, 1983; Bag, 1983; 1985). However, effect of heat treatment on the myoblast differentiation itself has not been examined.

In this study, for the evaluation of influence of hyperthermia on myogenesis, the effects of heat shock on the protein synthesis, proliferation, fusion, synthesis of creatine kinase, and change of cholesterol content in plasma membrane were investigated using the chick myogenic cells by treating heat at varying stages of myogenesis.

MATERIALS AND METHODS

Materials

12-day old hen's eggs were purchased from Yuil farm. RPMI 1640 medium, horse serum, and antibiotics(penicillin-streptomycin solution and fungizone) were obtained from Gibco, and ^{35}S-methionine(specific activity; 800 Ci/ml) from Amersham. All other chemicals were obtained from Sigma unless otherwise indicated.

Cell Culture

Myoblast cultures were prepared according to the method of O'Neill and Stockdale(1972) with minor modifications. Pectoral muscle was dissected from 12-day old chick embryo. After removing connective tissues, the muscle was minced, incubated in 0.125% trypsin and 0.001% DNase for 20 min at 37°C, and dispersed by repeated pipetting. Cells were centrifuged and suspended in 8110 medium(RPMI 1640 medium supplemented with 10% horse serum, 10% embryo extract, and 1% antibiotics). The cells were preplated on a collagen-coated dish for 10 min to remove fibroblasts and filtered through four layers of lens paper to remove undissociated cells. The cells were then plated at 4×10^5 cells/ml. Medium was changed after 24 hr and thereafter at 2-day intervals with 8102 medium (RPMI 1640 medium supplemented with 10% horse serum, 2% embryo extract, and 1% antibiotics). Plated cells were placed in a humidified incubator in the atmosphere of 95% air and 5% CO_2 at 37°C.

The collagen solution was made by autoclaving 0.5 mg of collagen per ml of distilled water followed by filtering through Millipore filter(pore size; 0.45 μm).

Preparation of Embryo Extract

Eye-balls, beaks and feet were removed from 12-day old chick embryos. The embryos were minced and homogenized by passing through a 50-ml syringe. The extract was diluted with RPMI 1640 medium and kept frozen at -70°C. Before use, the extract was centrifuged at 17,000 g for 1 hr and the supernatant was used as an embryo extract.

Exposure to Hyperthermia

Culture dishes which contain growing cells were sealed with parafilm and immersed into a constant-temperature circulating water bath for a heat shock. The medium was changed immediately before and after the heat treatment.

Measurement of Cell Fusion

Cells were fixed in ethanol and stained with Giemsa solution. The cell fusion was determined by direct microscopic examination at 400 X. The degree of fusion was determined

by the ratio of the number of nuclei within the myotubes of three or more nuclei divided by the total number of nuclei. Ten fields were randomly chosen for each dish.

Creatine Kinase Assay

Creatine kinase(CK) activity was estimated by the method of Koedam(1969). Dishes were washed with phosphate buffered saline(PBS) for three times and stored at -70°C until used for analysis. Cells were collected using a rubber policeman after the addition of Tris(100 mM)-magnesium(25 mM) buffer(pH 6.8). The samples were sonicated and centrifuged, and the supernatants were used for analysis. Reaction was started by adding ADP solution and stopped by $Ba(OH)_2$-EDTA solution (mixture of 1 volume of 5% $Ba(OH)_2$ and 2.5 volumes of 0.246% EDTA) and 5% $ZnSO_4$ soluticn. After centrifuging the samples, the supernatants were diluted with distilled water and ninhydrin solution (1%) was added. After the addition of KOH solution(10%) fluorescense was measured by Mark IV spectrofluorometer (excitation 405 nm; emission 520 nm).

DNA Estimation

DNA content was estimated by the methed of Kissane and Robins(1958). Calf thymus DNA was used as a standard. The samples were mixed with 0.1 ml of 3,5-diaminobenzoic acid(DABA; 40% w/v) and the mixtures were incubated at 60°C for 45 min. The reaction was stopped by the addition of 2 ml of 1 N HCl, and fluorescence was then measured(excitation 405 nm; emission 520 nm).

Determination of Cholesterol Content

Cholesterol content was determined by the method of Glick and Fell(1964). Briefly, cells in dishes were washed with PBS and harvested. Absolute alcohol and color reagent (2.5% $FeCl_3 \cdot 6H_2O$ in 86% H_3PO_4) were added and then absorbance was determined at 558 nm.

Gel Electrophoresis

Cells were labeled with 10 μCi of ^{35}S-methionine per ml of methionine-free RPMI 1640 at 37°C for 2 hr. After the labeling, cells were washed with cold PBS and harvested with the sample buffer (62.5 mM Tris-HCl, pH 6.8, containing 2% SDS and 10% glycerol). The samples were then electrophoresed in 10% (w/v) polyacrylamide gel in the presence of SDS as described by Laemmli (1970). The gels were then dried and exposed on Kodak X-Omat film. Molecular weight markers used were β-galactosidase (116,000), phosphorylase b (97,000), bovine serum albumin (68,000), egg albumin (45,000), and carbonic anhydrase (29,000). Proteins were estimated as described by Lowry *et al.* (1971) using bovine serum albumin as a standard.

^{35}S-Methionine Incorporation

Cells were labeled for 2 hr at 37°C with ^{35}S-methionine(10 μCi/ml). Cells were washed with PBS, harvested, and resuspended in the sample buffer used for SDS/polyacrylamide gel electrophoresis. The samples were stored at -20°C until used for analysis. Radioactivity of ^{35}S-labeled proteins was measured in a liquid scintillation spectrometer.

RESULTS

Effect of Heat Treatment on the Protein Synthesis during Myogenesis

To examine the pattern of protein synthesis in heat-treated myogenic cells, 24-hr old myoblasts or 96-hr old myotubes were treated with heat by shifting 1 hr from 37°C to a temperature between 42°C and 47°C. Cells were then labeled with ^{35}S-methionine at 37°C for 2 hr. Reduction in the overall protein synthesis after heat treatment was more severe in 24-hr old myoblasts than in 96-hr old myotubes. For instance, the overall protein synthesis in 24-hr old cells fell gradually after a shift to 42°C or above, whereas that in 96-hr old cells was not significantly altered by a shift to less than 45°C but fell sharply after a shift to higher than this temperature. Exposure of cells to temperatures above 43°C resulted in the induction of synthesis of five heat shock proteins having molecular weights of 96,000, 91,000, 80,000, 70,000, 69,000, and 25,000. Synthesis of heat shock proteins was most evident when the cells were incubated for 1 hr at 44~46°C(Fig. 1).

To examine if myogenic cells differ in the pattern of synthesis of heat-induced proteins as the cells progress differentiation, 24-hr, 72-hr, 96-hr, and 120-hr cultures were heat-shocked at 45°C for 1 hr and then labeled as above. No difference was apparent in the pattern of synthesis of HSPs throughout myogenesis (Fig. 2).

Effects of Actinomycin D and Cycloheximide on the Synthesis of Heat Shock Proteins

Actinomycin D or cycloheximide was added to media during heating at 45°C for 1 hr or during heating and incubation periods. After the treatments, the cells were incubated at

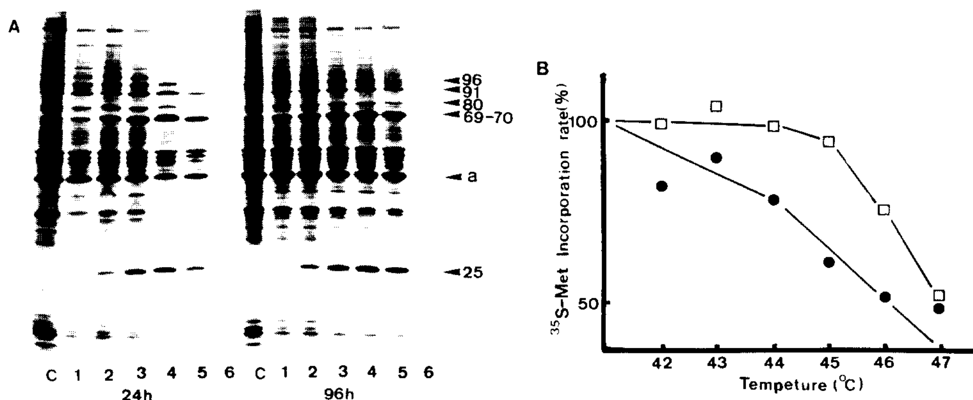

Fig. 1. Effect of hyperthermic treatment on protein synthesis in primary cultures of chick myoblasts and myotubes. 24-hr or 96-hr old cells were incubated at 42~47°C for 1 hr and labeled for 2 hr. (A) Autoradiograms of SDS/polyacrylamide slab gels of ^{35}S-methionine labeled proteins. Heat shock proteins are indicated by arrows with M_r's $\times 10^{-3}$ along with actin(a). C, unheated control; 42~47°, heated at the indicated temperature. (B) Relative levels of total protein synthesis in heated myoblasts and myotubes when protein synthesis in unheated control was taken as 100%. •-•, heated at 24 hr after cell seeding; □-□, heated at 96 hr after cell seeding.

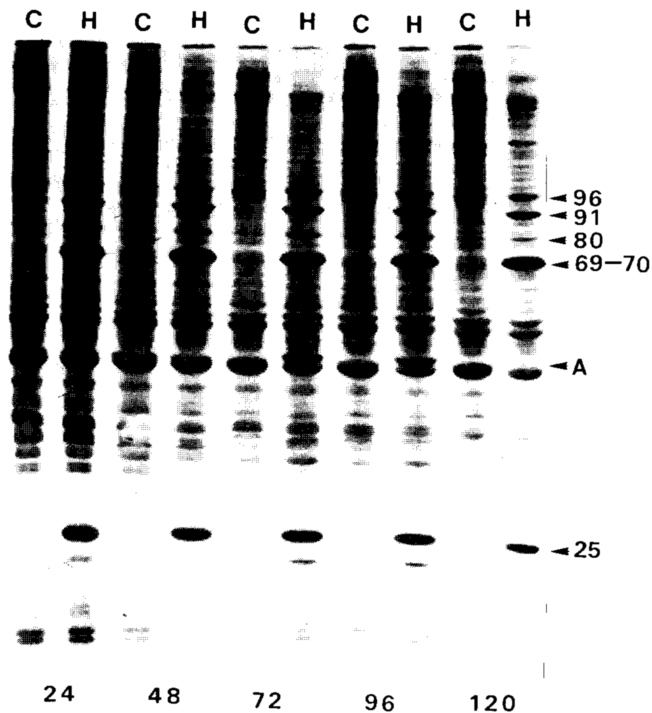

Fig. 2. Autoradiograms of SDS/polyacrylamide slab gels of proteins synthesized by control(C) and heat-shocked cells(H) at 45°C for 1 hr during myogenesis. Cells were heat-shocked at 24, 48, 72, 96, or 120 hr after cell seeding, then incubated at 37°C for 2 hr and labeled for 2 hr with ^{35}S-methionine. Heat shock proteins are indicated by arrows with M_r's$\times 10^{-3}$ along with major structural protein, actin(A), for comparison.

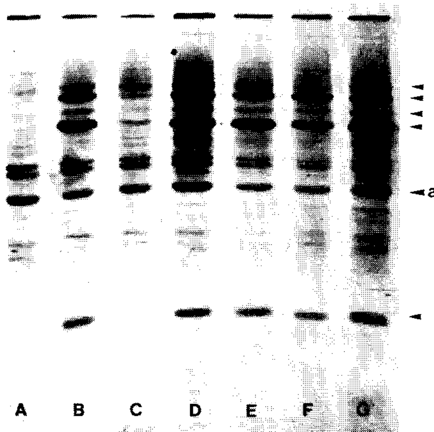

Fig. 3. Effects of actinomycin D(5 μg/ml) and cycloheximide(5μg/ml) on the synthesis of heat shock proteins in 24-hr old myoblasts treated at 45°C for 1 hr. After heat treatment, cells were allowed to recover at 37°C for 2 hr and then labeled for 2 hr. The labeled proteins were analyzed by autoradiographs of SDS/polyacrylamide slab gels. Heat shock proteins are indicated by arrows and 'a' indicates the position of actin. A, control; B, heated; C, actinomycin D during heat treatment; D, actinomycin D for first half of incubation period; E, actinomycin D for whole incubation period; F, cycloheximide during heat treatment; G, cycloheximide for heating and incubation periods.

37°C, and labeled for 2 hr. When actinomycin D was added during heat treatment, the synthesis of HSPs was blocked. However, the synthesis of HSPs was not blocked by cycloheximide(Fig. 3). Thus, the synthesis of HSPs is likely to be regulated at the level of transcription and not of translation.

Effects of Heat Treatment on Myogenesis

To observe effects of hyperthermia on the cell differentiation, proliferation, fusion index, and creatine kinase activity of heat-treated cells were compared with those of unheated control cells. After heat treatment at 45°C for 1 hr, cell proliferation was slightly reduced in 24-hr cultures but not in 55-hr cultures(Fig. 4). When 24-hr old myoblasts were exposed to hyperthermia at 45°C for 1 hr, increase in creatine kinase activity as well as

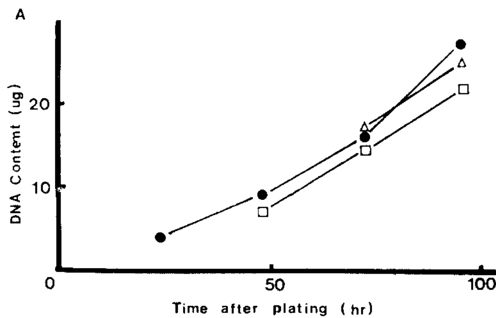

Fig. 4. Effect of heat treatment on myoblast proliferation. Cells were heated at 45°C for 1 hr at 24 hr or 55 hr after cell seeding. After harvested with TM buffer, DNA content was investigated. ●-●, control; □-□, heated at 24 hr; △-△, heated at 55 hr.

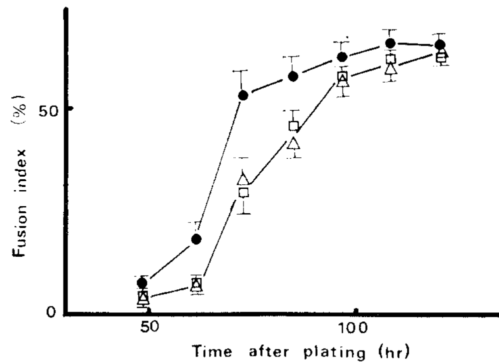

Fig. 5. Effect on fusion index of heat treatment at 45°C for 1 hr to 24-hr old myoblast. ●-●. control; □-□, heated once; △-△, heated twice with 4 hr interval between.

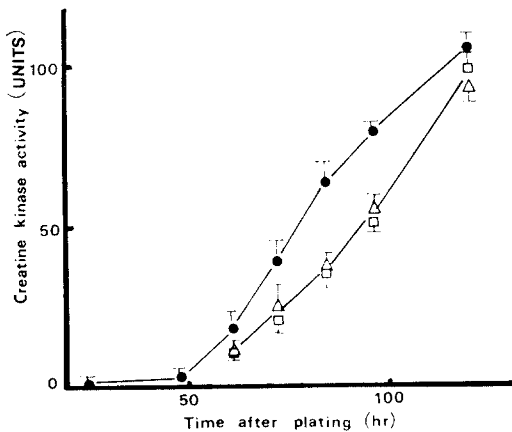

Fig. 6. Effect on creatine kinase activity of heat treatment at 45°C for 1 hr to 24-hr old myoblasts. ●-●, control; □-□, heated once; △-△, heated twice with 4 hr interval between.

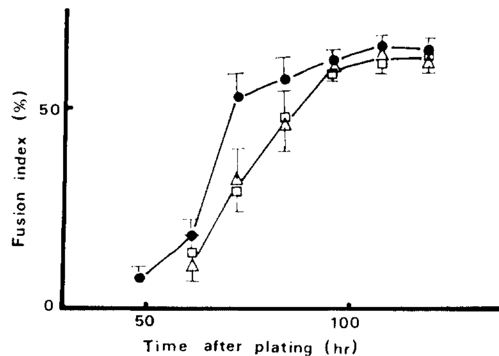

Fig. 7. Effect on fusion index of heat treatment at 45°C for 1 hr to 55-hr old cells. ●-●, control; □-□, heated once; △-△, heated twice with 4 hr interval between.

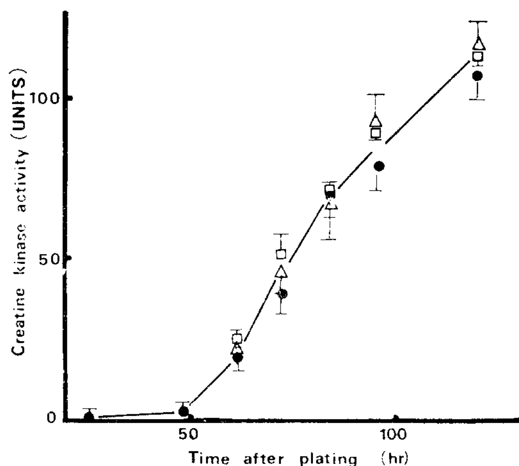

Fig. 8. Effect on creatine kinase activity of heat treatment at 45°C for 1 hr to 55-hr old cells. •-•, control; □-□, heated once; △-△, heated twice with 4 hr interval between.

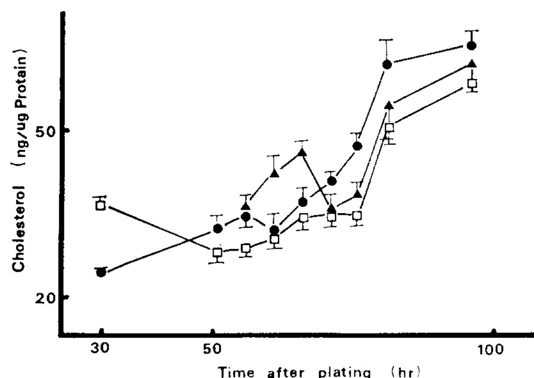

Fig. 9. Effect of heat treatment at 45°C for 1 hr on the cholesterol content in plasma membrane. Hyperthermia was administered at 24 hr or 55 hr after cell plating and heated cells were then incubated at 37°C. •-•, control; □-□, heated at 24 hr; ▲-▲, heated at 55 hr.

cell fusion was delayed(Figs. 5 and 6). When the same heat treatment was given to 55-hr old cells, however, cell fusion was delayed but creatine kinase activity appeared without delay(Figs. 7 and 8). These results show that there may be a significant difference between 24-hr and 55-hr old cells in response to heat shock.

Change of Cholesterol Content in Plasma Membrane

Unheated control cells underwent a change in the cholesterol content in plasma membrane during myogenesis. At 24 hr after cell seeding, cholesterol content was about 25 ng/μg protein. During cell fusion, it remained unchanged at about 35 ng/μg protein. It then increased as the myotubes were mature. To examine the effect of heat shock on the change of cholesterol content, cultured cells were exposed to 45°C for 1 hr at 24 hr or 55 hr after cell seeding. As shown in Fig. 9, heat treatment induced a transient increase in cholesterol content at about 6 hr after the heat shock. Cholesterol level was then increased in a similar fashion to the control cells. Incidently, when the 55-hr cultures were incubated at 43°C for 1 hr, there was no difference between control and heat-treated cells in the pattern of change in cholesterol content. In the later case, no change in the fusion index was observed(Fig. 10). These results suggest that the transient increase in cholesterol content in 55-hr heat-treated cells at 45°C is responsible for the delay in cell fusion.

Kinetics for the Heat Shock Protein Synthesis following Hyperthermia

24-hr or 96-hr old cells were heat-shocked at 45°C for 1 hr and incubated at 37°C for 0 to 24 hr followed by labeling for 2 hr. All heat-induced proteins were synthesized actively up to 6 hr after heating (Fig. 11).

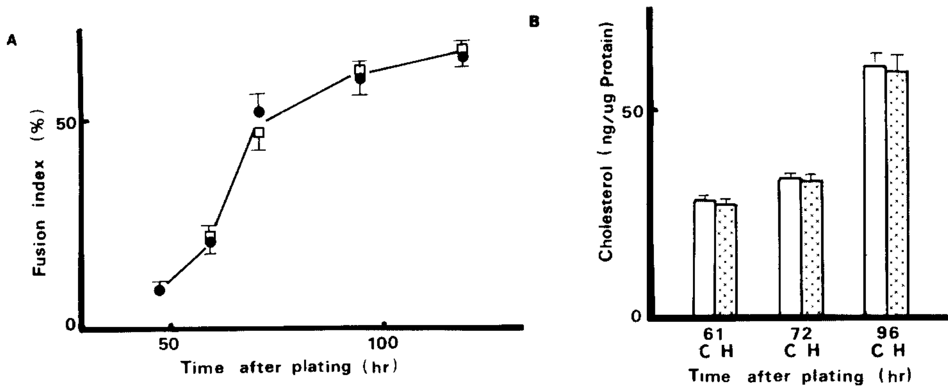

Fig. 10. Effects on fusion index and cholesterol content in plasma membrane of heat treatment at 43°C for 1 hr to 55-hr old cells. (A) Fusion index. •-•, control; □-□, heated (B) Cholesterol content. C, control; H, heated.

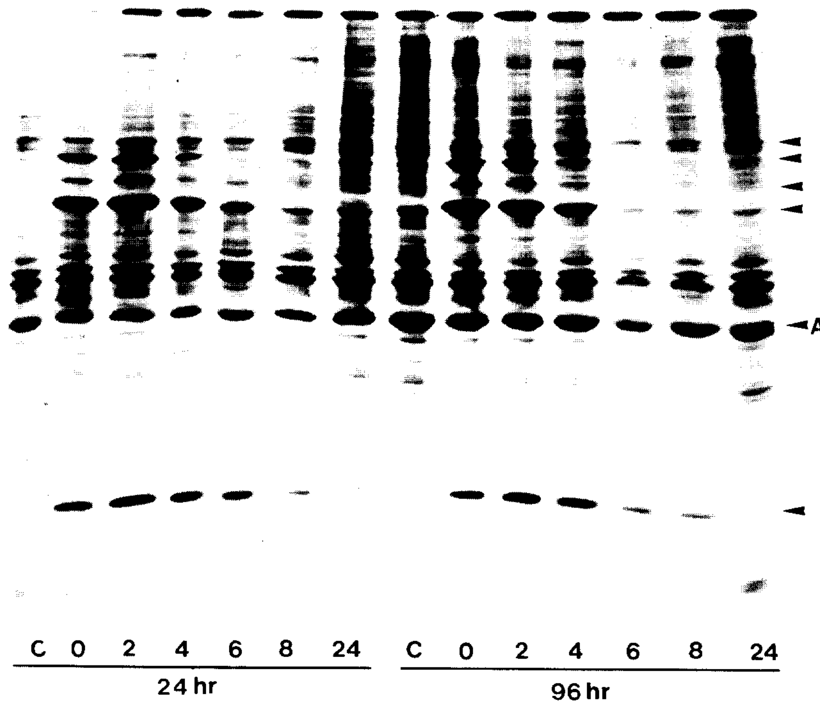

Fig. 11. Kinetics for the synthesis of heat shock proteins following hyperthermia. Cells were exposed to 45°C for 1 hr at 24 hr or 96 hr after cell plating and then incubated at 37°C for 0~24 hr before labeling for 2 hr. The labeled proteins were analyzed by autoradiographs of SDS/polyacrylamide slab gels. The arrows indicate HSPs and 'A' indicates actin band. C, unheated control; 0~24. time of incubation before labeling.

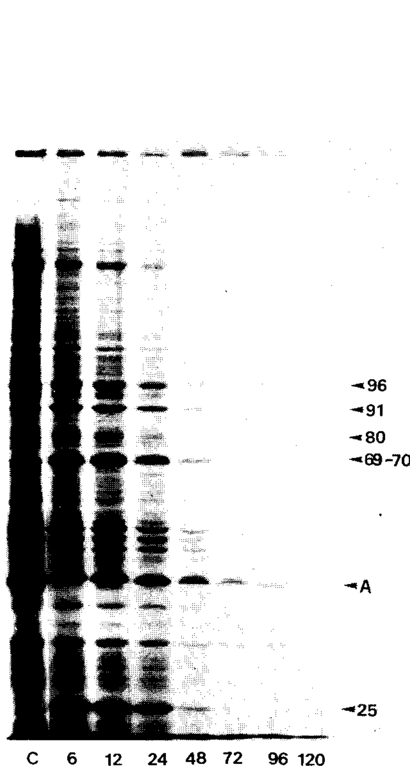

Fig. 12. Decay pattern of heat shock proteins. After the heat treatment at 45°C for 1 hr to 24-hr old myoblasts, the cells were incubated at 37°C for 4 hr, labeled for 2 hr, and then incubated at 37°C for additional 0~114 hr before harvesting. Heat shock proteins are indicated by arrows with M_r's$\times 10^{-3}$ and 'A' indicates actin. C, unheated control; 6~120, time of incubation after heat treatment.

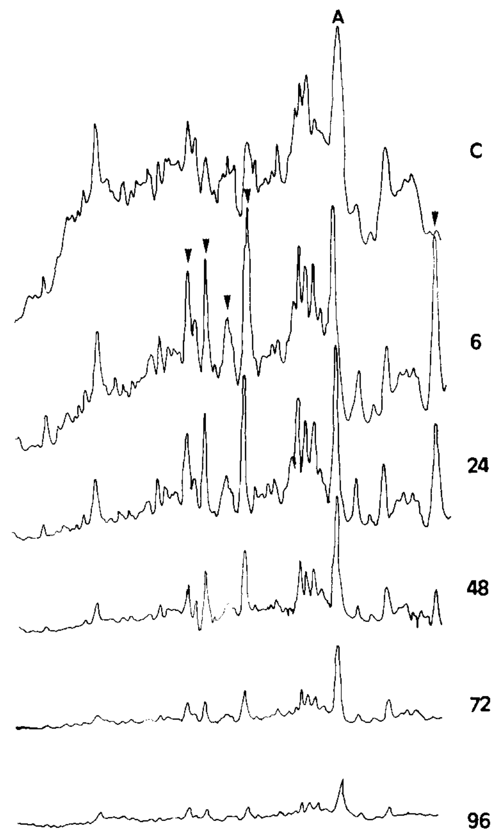

Fig. 13. Densitometric scan of the autoradio graphed films shown in Fig. 12. Heat shock proteins are indicated by arrows along with major structural protein, actin(A) for comparison.

Decay of Synthesized Heat Shock Proteins

Cells were exposed to 45°C for 1 hr at 24 hr after cell plating, incubated at 37°C for 4 hr, and then labeled for 2 hr. Cells were then washed with medium and incubated at 37°C up to 120 hr. As shown in Figs. 12 and 13, heat-treated cells conserved HSPs up to 72 hr after heating.

Effects of Double Heat Treatments on the Myogenesis and Heat Shock Protein Synthesis

After an initial heat treatment at 45°C for 1 hr to 24-hr or 55-hr cultures, cells were incubated at 37°C for 4 hr and treated again with the same heat shock as the first. As shown in Figs. 5 to 8, little or no difference in the fusion index and creatine kinase activity was observed in the cells either heated once or twice as well as either heated at

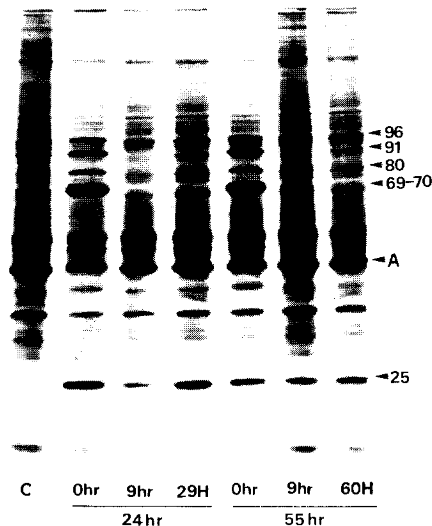

Fig. 14. Effect of second heat treatment on the synthesis of heat shock proteins. 24-hr or 55-hr old cells were exposed to 45°C for 1 hr once and labeled immediately or after 9 hr incubation, or heat-shocked twice with 4 hr interval between and labeled after 4 hr incubation. The labeled proteins were analyzed by autoradiographs of SDS/polyacrylamide slab gels. C, control; 0 hr, labeled immediately; 9 hr, labeled after 9 hr incubation; 29 H or 60 H, heated twice.

24-hr or 55-hr after seeding.

To examine the synthesis of HSPs after the second shock, 24-hr or 55-hr old cells were treated once with heat and labeled immediately or after 9 hr incubation, or heated twice and labeled after 4 hr incubation (Fig. 14). The heat-induced proteins, except 25, 000 dalton protein, were not induced by the second heat treatment. These data indicate that the cells acquire thermotolerance as a consequence of the heat exposure preceeding the second.

DISCUSSION

It has been reported that differentiation of myoblasts are not only determined by the intrinsic program in myoblasts, but can easily be controlled by simple environmental manipulations. Up to now, a number of chemicals which exert an influence on differentiation of myoblasts have been reported (Gilfix and Sanwal, 1980; Couch and Strittmatter, 1983; Den *et al.* 1975; Nameroff and Munar, 1976; Wakelam and Pette, 1982; Kang *et al.*, 1983). Nevertheless, it is still uncertain how myoblasts differentiate.

Atkinson (1981) reported the synthesis of heat shock proteins by quail myoblasts undergoing myogenesis. Quite recently, Bag (1983; 1985) examined the recovery of normal protein synthesis in heat-shocked chicken myotubes. In addition, it was reported that arsenate induced heat shock proteins in cultured rat myoblasts (Kim *et al.*, 1983). However, the effect of hyperthermia on the differentiation of myoblasts is largely unknown.

In the present study, the influence of hyperthermia on morphological and biochemical

differentiations of myoblasts was examined. By heat treatment at 45°C for 1 hr, increase of creatine kinase activity as well as cell fusion was delayed in 24-hr old cells, whereas in 55-hr old cells cell fusion alone was delayed. 24-hr old myoblasts divide rapidly but display few other features at this stage that distinguish them from fibroblasts. 55-hr old cells, called presumptive myoblasts, are withdrawn from cell cycle, no longer undergo mitosis and participate in the recognition or clustering of myoblasts at the same stage of differentiation. Many studies have revealed that in the cells cultured with a low calcium media by using Chelex medium or by treatment of EGTA, cell fusion was blocked but an increase of muscle-specific protein synthesis continued(Turner *et al.*, 1976; Moss and Strohman, 1976). Dym *et al.* (1978) also showed that inhibition of RNA synthesis by actinomycin D (2 ng/ml) added shortly before the onset of cell fusion did not prevent the increase in creatine kinase activity. Since actinomycin D was added prior to the main phase of cell fusion, this result supports that in differentiating muscle cultures the gene encoding creatine kinase can be activated some time prior to cell fusion and that the regulation of synthesis of creatine kinase may occur at posttranscriptional level. In these regards, it can be suggested that cell fusion is influenced in presumptive myoblasts(24-hr cultures) as well as proliferative myoblasts(55-hr cultures) by hyperthermia, whereas the synthesis of creatine kinase is not influenced in presumptive myoblasts in which the gene of creatine kinase seems to have been activated prior to hyperthermia. Referring to these data, it is likely that the synthesis of muscle-specific proteins is not directly dependent on the fusion of myogenic cells undergoing myogenesis.

Cholesterol is a primary component of the mammalian cell plasma membrane and plays a role in maintaining membrane fluidity and rigidity. Cress and Gerner(1980) reported that cholesterol levels inversely reflected the thermal sensitivity of mammalian cells in culture. In the present study, a transient increase in cholesterol content has shown to be induced by heat treatment. Furthermore, delay of cell fusion was shown to be consistent with the temporary increase in cholesterol content at 55 hr after cell seeding. It has also been found that cell fusion was not delayed and cholesterol content was not changed in the 55-hr old cells heated at 43°C for 1 hr. In 1977, Prives and Shinitzky revealed that an increased membrane fluidity preceded the fusion of muscle cells. Thus, it is likely that heated 55-hr old muscle cells increase in their cholesterol content in plasma membrane to mininize the effects of heat shock and this increase in cholesterol content may consequently inhibit cell fusion by causing a change in the fluidity of plasma membrane.

Like many other cells, myogenic cells following hyperthermia could develop thermotolerance, i.e. a transient increased tolerance to subsequent thermal treatment. The development of a transient thermotolerance following sublethal heat shock was reported to be correlated with the synthesis of HSPs(Landry *et al.*, 1982; Li and Werb, 1982; McAlister *et al.*, 1980; Muller *et al.*, 1985). Accordingly, heat-shocked myogenic cells did not induce an additional synthesis of HSPs by the second heat treatment. In addition, there was no

difference in the fusion index and creatine kinase activity, even if the cells were exposed to a second heat.

Finally, it seems likely that there is little difference in kinds of heat-induced proteins in the cells at varing stages of chicken myogenesis. In other words, when myogenic cells were heated at 45°C for 1 hr at varying stages after cell seeding, they synthesized the same kinds of heat-induced proteins, ie., 96,000, 91,000, 80,000, 70,000, 69,000, and 25,000 daltons. This result is not in agreement with that of Atkinson(1981), in that heat shocked myotubes did not synthesize detectable levels of the 25,000 dalton polypeptide which was found in the heat shocked myoblasts. This difference may be ascribed to the different origins of myoblasts(chicken embryos and quail embryos). This result suggests that the HSPs are conserved well in the course of evolution. It was also shown that HSPs were synthesized actively up to 6 hr after hyperthermia and were degraded slowly through 72 hr. Although the exact function of heat shock proteins is still obscure, many studies have suggested that heat-induced proteins, in general, function to maintain cellular homeostasis during cellular trauma(Ashburner *et al.*, 1979). Thus, heat shock proteins may play a role in providing thermotolerance for the heated cells rather than affecting the myoblast differentiation.

In conclusion, the present study has shown that hyperthermia influences the myoblast differentiation probably by affecting several targets, such as gene expression, plasma membrane. and so forth. Therefore, the effect of hyperthermia was different as to the stages of differentiation at which the cells are heat-shocked. Also confirmed by the present study is that chicken myogenic cells acquire the general characteristics, the synthesis of HSPs and thermotolerance which go with hyperthermia.

ABSTRACT

To investigate the effect of hyperthermic treatment on myoblast differentiation *in vitro*, protein synthesis, myoblast proliferation, fusion index, creatine kinase activity, and cholesterol content in plasma membrane were examined following heat treatment at varying stages of myogenesis.

Cell proliferation was slightly affected by hyperthermia. Cell fusion and an abrupt increase of creatine kinase activity were delayed by heat treatment in 24-hr old cells at 45°C for 1 hr, whereas cell fusion alone was delayed in 55-hr old cells. In addition, a transient increase of cholesterol content in plasma membrane was detected following hyperthermia. These results revealed that effects of hyperthermia on myoblast differentiation differed with varying stages in myogenesis and suggested a possibility that a transient increase of cholesterol content exert an influence on the cell fusion.

Heat treatment at 43°C or higher induced the synthesis of a family of proteins called 'heat shock proteins' regardless of stages of myoblast differentiation and reduced the syn-

thesis of proteins produced at normal temperature. The induction of synthesis of heat shock proteins was blocked by treatment of actinomycin D but not by cycloheximide.

Cell fusion and creatine kinase activity were not altered to a measurable extent by double heat treatments of the same intensity and the second heat treatment did not induce measurable additional synthesis of HSPs, probably due to the increased resistance to hyperthermia as a consequence of the initial heat treatment.

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