Possible Involvement of Phosphorylated Heat-Shock Factor-1 in Changes in Heat Shock Protein 72 Induction in the Failing Rat Heart Following Myocardial Infarction

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It is supposed that an increase in the level of heat shock protein 72 (HSP72) in the failing heart would be beneficial for reducing the myocardial damage. However, the induction of HSP72 after an exposure to heat shock is blunted in the failing rat heart following myocardial infarction. In this study, to clarify the possible mechanisms underlying this reduction in the ability for HSP72 induction in the failing heart, the possible involvement of heat-shock factor-1 (HSF1), an HSP transcription factor, in this reduction was examined. When hemodynamic parameters of rats with myocardial infarction 8 weeks after coronary artery ligation were measured, the animals showed the signs of chronic heart failure. The HSF1 content was increased in the viable myocardium in the failing heart. The ability to induce cardiac HSP72 was reduced after an exposure to hyperthermia. The level of HSF1 in the cytosolic fraction from the failing heart with or without exposure to hyperthermia was increased, whereas that of HSF1 in the nuclear fraction was reduced. In the failing heart, the level of HSF1 on its serine 303 (Ser303) residue, which phosphorylation represses HSF1, was increased. These findings suggest that HSF1 translocation from the cytosol into the nucleus was attenuated after an exposure to hyperthermia and that an increase in the phosphorylation of HSF1 Ser303 was involved in the impairment of heat shock-induced HSP72 induction in the failing heart following myocardial infarction.

Key words chronic heart failure; heat shock protein; heat-shock factor-1; myocardial infarction

Heart failure represents a final common endpoint for various heart diseases, leading to cardiovascular death. Heart failure is also considered as a chronic state of inflammation and stress on the heart tissue. Heat shock provides a protective effect on the ischemic heart via the enhanced synthesis of several heat shock proteins (HSPs). In acute myocardial infarction, HSP, especially HSP72, is rapidly expressed to afford tolerance against ischemic myocardial injury. Therefore, it is assumed that an increase in HSP72 in the failing heart would be beneficial for reducing the myocardial damage. However, our previous study showed that the induction of HSP72 following heat shock is blunted in the failing heart following myocardial infarction.

Numerous studies reveal that cellular upregulation of the stress-inducible isoform within the 70-kDa family of HSPs, HSP72, occurs in response to heat stress, oxidative stress, pH disturbances, and exercise. The protective effect of HSP72 is potentially mediated by its molecular chaperon function, which is one of several functional properties of HSP72, including active participation in folding proteins by minimizing incorrect interactions within and between molecules, maintaining proteins in their native folded state, and in repairing or promoting the degradation of misfolded proteins.

HSP72 expression is predominantly regulated by the transcription factor known as heat-shock factor-1 (HSF1). HSF1 remains as a latent monomeric protein in the cytoplasm under unstressed conditions. When HSF1 is activated, serine residue 230 of HSF1 in the cytosol is phosphorylated and then the transcription factor is transferred into the nucleus, where it binds to DNA. In contrast, phosphorylation on Ser303 in HSF1 by glycogen synthase kinase 3β (GSK3β) reportedly has a negative regulatory effect on HSF1 activation. However, the phosphorylation state and subcellular localization of HSF1 in the failing heart are unknown yet.

In this study, the phosphorylation and subcellular localization of HSF1 in the failing rat heart were examined to clarify the mechanism of the impairment of HSP72 induction following heat shock.

MATERIALS AND METHODS

Animals and Operation Male Wistar rats (SLC, Shizuoka, Japan), weighing 220–240 g, were used in the present study. The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996). The protocol of the present study was approved by the Committee of Animal Use and Welfare of Tokyo University of Pharmacy and Life Sciences.

Myocardial infarction of rats was produced by ligation of the left ventricular (LV) coronary artery according to the method described previously. Rats with myocardial infarction (coronary artery ligation (CAL) rats) with approximately 40% infarct area in the left ventricle are consistently produced under our experimental conditions. Sham-operated rats (Sham rats) were treated in a similar manner except no coronary artery ligation was performed.

Echocardiographic Measurements Two and eight weeks after the operation, CAL (2W-CAL and 8W-CAL) and Sham (2W-Sham and 8W-Sham) rats were anesthetized with 40 mg/kg intraperitoneally (i.p.) pentobarbital sodium, and then their chest hair was shaved off before examination. Two-dimensional and Doppler imaging were performed by using a ProSound 5500® (Aloka, Tokyo, Japan) equipped with a 10-MHz transducer. The transthoracic echocardiographic probe was placed so as to obtain short-axis and long-axis views. The LV inter-

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nal diameters at end diastole (LVIDd) and systole (LVIDs) were measured, and then the LV fractional shortening (FS) and ejection fraction (EF) were calculated from the LV dimensions. The cardiac output (CO) and stroke volume (SV) at the pulmonary artery and the Tei index were measured based on the long-axis and apical four-chamber views. After determination of the pulmonary arterial flow, heart rate (HR), velocity time integral (VTI), and pulmonary arterial diameter (PAD) were measured by using the long-axis view; and the ratios of CO and SV to body weight (BW) were calculated as COI and SVI, respectively.

Invasive Measurement of Hemodynamic Parameters
After measurement of cardiac functions by echocardiography, hemodynamic parameters of the CAL (2W-CAL and 8W-CAL) and Sham (2W-Sham and 8W-Sham) rats that had been anesthetized with pentobarbital sodium (40 mg/kg i.p.) were collected for 10 min. Western blotting analysis was performed according to the method described previously with some modifications.17) The heart was isolated and divided into LV wall, septum (Sep), and right ventricular (RV) wall, and ejection fraction (eF) were calculated from the LV dimensions at end diastole (LVIDd) and systole (LVIDs) were measured, and then the LV fractional shortening (FS) and ejection fraction (EF) were calculated from the LV dimensions. The cardiac output (CO) and stroke volume (SV) at the pulmonary artery and the Tei index were measured based on the long-axis and apical four-chamber views. After determination of the pulmonary arterial flow, heart rate (HR), velocity time integral (VTI), and pulmonary arterial diameter (PAD) were measured by using the long-axis view; and the ratios of CO and SV to body weight (BW) were calculated as COI and SVI, respectively.

Heat Shock Treatment for Rats
Sham and CAL rats were anesthetized with pentobarbital sodium (40 mg/kg i.p.) and then submerged in a water bath at 42°C. Respiration was maintained through tubes connecting the trachea to the respirator. The body temperature of the animals was allowed to reach 42°C and was thereafter maintained at that body temperature for 15 min in the water bath.

Western Blotting and Detection of Proteins
Sample preparation for Western blotting was performed according to the method of Takahashi et al.18) The heart was isolated and divided into LV wall, septum (Sep), and right ventricular (RV) wall; and then these tissues were weighed. Furthermore, the isolated LV wall was separated into viable area (viable LV) and scar tissue and stored at −80°C. The viable LV was homogenized in a homogenization buffer (250 mm sucrose, 20 mm 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES), 1 mm dithiothreitol (DTT), 1 mm ethylene glycol-bis(2-aminoethyl)ether)-N,N,N',N'-tetraacetic acid (EGTA), Complete® protease inhibitor cocktail (Roche, Basel, Switzerland), and PhosSTOP® phosphatase inhibitor cocktail (Roche); pH 7.4, at 4°C). The homogenate was centrifuged at 1000×g for 10 min, the pellet was collected as the crude nuclear fraction. The resultant supernatant fluid was centrifuged at 10000×g for 1 h and then the resulting supernatant was collected as the cytosolic fraction. The resuspended-cruude nuclear fraction was added 10% Triton X-100 for a final concentration of 0.5%, and this mixture was incubated for 30 min at 4°C. Then, the fluid was filtered and centrifuged at 10000×g for 10 min. Western blotting analysis was performed according to the method described previously with some modifications.18) Anti-HSP72/73 mouse monoclonal (W27, Merck Billerica, MA, U.S.A.), anti-HSF1 rabbit polyclonal (Enzo Life Science, Inc., Farmingdale, NY, U.S.A.), anti-phospho HSF1 (Ser303) rabbit polyclonal (GenScript, Piscataway, NJ, U.S.A.), anti-Akt rabbit polyclonal (Cell Signaling Technology, Inc., Danvers, MA, U.S.A.), anti-phospho Akt (Ser473) rabbit polyclonal (Cell Signaling Technology), anti-GSK3α/β mouse monoclonal (1H8, Enzo Life Science), anti-phospho GSK3β (Ser9) rabbit polyclonal (Cell Signaling Technology), anti-histone H1 rabbit polyclonal (Sigma-Aldrich Corp., St. Louis, MO, U.S.A.), anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mouse monoclonal (H6C5, Merck), and anti-actin mouse monoclonal (HHF35, Sigma-Aldrich Corp.) antibodies were used. Detection and quantification of these proteins on polyvinylidene difluoride (PVDF) membranes were performed by the method described previously.18)

Immunohistochemical Analysis
Hearts were quickly excised and fixed by coronary perfusion with 4% paraformaldehyde in 0.1 m phosphate buffer. The fixed tissue was incubated in 30% sucrose solution and then embedded in Neg-50 (Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.). Cryosections prepared by using a Microm HM550 cryostat (Thermo Fisher Scientific Inc.) were incubated with 1% Triton X-100 in phosphate buffered saline (PBS) for 1 h. Then, the slices were washed with PBS and incubated with a blocking solution (Blocking-one, Nacalai Tesque, Kyoto, Japan). After blocking, the slices were incubated with anti-HSF1 rabbit polyclonal (Enzo Life Science) overnight at 4°C. Then the slices were washed and incubated with anti-α-actinin mouse monoclonal (Sigma-Aldrich Corp.), and subsequently with Alexa488 (Life Technologies Co., Carlsbad, CA, U.S.A.) or Cy3-conjugated secondary antibodies (GE Healthcare U.K. Ltd., Little Chalfont, U.K.). Fluorescent images were captured by a CCD camera (DP50, Olympus, Tokyo, Japan) mounted on an Olympus BX52 microscope equipped with a mercury arc lamp.

Statistics
The results were expressed as the means±S.E.M. Statistical significance of differences were estimated by using 2-way analysis of variance followed by Bonferroni/Dunn multiple comparisons (StatView version 5.0; SAS Institute Inc., Cary, NC, U.S.A.). Differences with a probability of 5% or less were considered to be significant (p<0.05). Two-side tests of significance were performed for all analyses.

RESULTS

Changes in Tissue Weight
Table 1 shows the changes in the tissue weight of the 2W-CAL and 8W-CAL rats. The LVW/BW ratios of the 2W and 8W-CAL rats were not significantly different from those of the corresponding Sham groups. The SepW/BW ratios of the 2W and 8W-CAL rats were increased to approximately 120% and 175%, respectively, of those of the corresponding Sham rats. Similarly, RVW/BW ratios and LungW/BW ratios of the 2W and 8W-CAL rats were increased as compared with those of the corresponding Sham groups. The RVW/BW ratios of the 2W and 8W-CAL rats were increased to approximately 180% and 290%, respectively, of the values for the corresponding Sham rats. The LungW/BW ratios of the 2W and 8W-CAL rats were increased to approximately 270% and 370% of the respective values for the corresponding Sham rats.

Changes in Echocardiographic Parameters
Table 2 shows the cardiac parameters determined at the 2nd and 8th weeks after the surgery, as assessed by the echocardiographic
### Table 1. Changes in Tissue-Weight Parameters of the Sham-Operated (Sham) Rats and Coronary Artery-ligated (CAL) Rats at the 2nd (2W) and 8th (8W) Weeks after Surgery

<table>
<thead>
<tr>
<th></th>
<th>2W</th>
<th>8W</th>
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<tbody>
<tr>
<td><strong>BW (g)</strong></td>
<td>242±3</td>
<td>224±3*</td>
</tr>
<tr>
<td><strong>LWV (mg)</strong></td>
<td>339±9</td>
<td>328±14</td>
</tr>
<tr>
<td><strong>LWV/BW (mg/g)</strong></td>
<td>1.4±0.04</td>
<td>1.46±0.06</td>
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<tr>
<td><strong>SepW (mg)</strong></td>
<td>134±4</td>
<td>153±7</td>
</tr>
<tr>
<td><strong>SepW/BW (mg/g)</strong></td>
<td>0.55±0.01</td>
<td>0.68±0.03</td>
</tr>
<tr>
<td><strong>RVW (mg)</strong></td>
<td>129±5</td>
<td>208±9*</td>
</tr>
<tr>
<td><strong>RVW/BW (mg/g)</strong></td>
<td>0.53±0.02</td>
<td>0.92±0.04*</td>
</tr>
<tr>
<td><strong>HW (mg)</strong></td>
<td>601±11</td>
<td>689±12*</td>
</tr>
<tr>
<td><strong>HW/BW (mg/g)</strong></td>
<td>2.48±0.04</td>
<td>3.06±0.04*</td>
</tr>
<tr>
<td><strong>LungW (mg)</strong></td>
<td>809±12</td>
<td>2006±87*</td>
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<tr>
<td><strong>LungW/BW (mg/g)</strong></td>
<td>3.35±0.07</td>
<td>8.93±0.44*</td>
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Each value represents the mean±S.E.M. of 8 independent experiments. *p<0.05 vs. the corresponding Sham group. **p<0.05 vs. 2W CAL group.

### Table 2. Changes in the Echocardiographic Parameters of the Sham-Operated (Sham) Rats and Coronary Artery-ligated (CAL) Rats at the 2nd (2W) and 8th (8W) Weeks after Surgery

<table>
<thead>
<tr>
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<th>8W</th>
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<tbody>
<tr>
<td><strong>LVDD (mm)</strong></td>
<td>5.63±0.09</td>
<td>9.04±0.15*</td>
</tr>
<tr>
<td><strong>LVDS (mm)</strong></td>
<td>2.83±0.08</td>
<td>7.39±0.13*</td>
</tr>
<tr>
<td><strong>FS (%)</strong></td>
<td>49.9±0.8</td>
<td>18.8±0.9*</td>
</tr>
<tr>
<td><strong>ESV (µL)</strong></td>
<td>23.1±1.8</td>
<td>395±20.5*</td>
</tr>
<tr>
<td><strong>EDV (µL)</strong></td>
<td>179±8</td>
<td>741±39*</td>
</tr>
<tr>
<td><strong>EF (%)</strong></td>
<td>87.3±0.6</td>
<td>46.2±1.8*</td>
</tr>
</tbody>
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### Table 3. Changes in the Hemodynamic Parameters of the Sham-Operated (Sham) Rats and Coronary Artery-ligated (CAL) Rats at the 2nd (2W) and 8th (8W) Weeks after Surgery

<table>
<thead>
<tr>
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<tbody>
<tr>
<td><strong>LVSP (mmHg)</strong></td>
<td>142±2</td>
<td>136±2*</td>
</tr>
<tr>
<td><strong>LVEDP (mmHg)</strong></td>
<td>2.2±0.4</td>
<td>22.5±2.4*</td>
</tr>
<tr>
<td><strong>LV +dp/dt (mmHg/s)</strong></td>
<td>10667±467</td>
<td>8116±379*</td>
</tr>
<tr>
<td><strong>LV −dp/dt (mmHg/s)</strong></td>
<td>−9270±267</td>
<td>−6359±368*</td>
</tr>
<tr>
<td><strong>RVSP (mmHg)</strong></td>
<td>31±1</td>
<td>56±2*</td>
</tr>
<tr>
<td><strong>RVEDP (mmHg)</strong></td>
<td>0.95±0.36</td>
<td>3.18±0.52*</td>
</tr>
<tr>
<td><strong>RV +dp/dt (mmHg/s)</strong></td>
<td>2191±52</td>
<td>3687±193*</td>
</tr>
<tr>
<td><strong>RV −dp/dt (mmHg/s)</strong></td>
<td>−1543±62</td>
<td>−2429±234*</td>
</tr>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td>109±3</td>
<td>107±4</td>
</tr>
<tr>
<td><strong>HR (bpm)</strong></td>
<td>423±6</td>
<td>400±8</td>
</tr>
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</table>

Each value represents the mean±S.E.M. of 8 independent experiments. *p<0.05 vs. the corresponding Sham group. **p<0.05 vs. 2W CAL group.
The left ventricular FS values, a measure of left ventricular systolic function, of the 2W and 8W-CAL rats were decreased to approximately 38% and 22%, respectively, of those for the corresponding Sham rats. The CO index of the 2W-CAL group was similar to that of the 2W-Sham group, whereas this value for the 8W-CAL group was decreased to approximately 72% of that for the corresponding Sham group. PAAT, an estimate of the pulmonary arterial systolic pressure, was decreased significantly at the 2nd and 8th weeks after the surgery. The LV and RV Tei indices, increases in which result from the development of LV dysfunction and provide prognostic information about a variety of myocardial conditions, were increased at the 2nd weeks after the surgery as compared with those for the corresponding Sham rats; and these values for the 8W-CAL group were further increased over those for the 2W-CAL group.

**Changes in Hemodynamic Parameters** Hemodynamic parameters of the Sham and CAL rats were determined at the 2nd and 8th weeks after the surgery by use of the cannula method (Table 3). The LVEDP of the CAL rats was increased at the 2nd week, and then further increased to more than 30mmHg at the 8th week. The RVSP of the 2W-CAL rats was increased to approximately 180% of the corresponding Sham value. The RVSP of the 8W-CAL rat was further increased to 2-fold of the 8W-Sham value. The heart rate did not change throughout the experiment. There were no changes in these hemodynamic parameters of the Sham rats throughout the experiment, when these values were compared with those for the control rats.

**Changes in the Basal Expression Level of HSP72 and**

Fig. 1. Changes in the Ability of the Left Ventricle to Induce HSP72 in the Sham-Operated (Sham) Rats and Coronary Artery-Ligated (CAL) Rats at the 2nd (2W) and 8th (8W) Weeks after Surgery

(A) HSP72 contents in the Sham and CAL animals before heat-shock treatment. (B) Time course changes in HSP72 contents following heat-shock treatment in the Sham and CAL rats. (C) HSP72 contents at 24h following heat shock treatment in the Sham and CAL rats. Each value represents the mean±S.E.M. of 6 independent experiments. *p<0.05 vs. the corresponding Sham group. †p<0.05 vs. 2W CAL group. ‡p<0.05 vs. the control group. ††p<0.05 vs. 2W CAL+HS group.
Its Induction Ability  The HSP72 content was measured by western blotting (Fig. 1). The HSP72 content in the 2W-CAL rats was increased to approximately 140% of that for the 2W-Sham rat. In the failing heart, the HSP72 content had the same value as that of the 8W-Sham animal (Fig. 1A). To examine the ability for induction of HSP72 in the heart, we preliminarily measured the time course changes in myocardial HSP72 contents in Sham and CAL rats after an exposure to HS. The HSP72 content reached its maximum level at 24 h after HS treatment and then decreased at 48 h in all groups except the 8W-CAL group, which showed no increase. The western blot for all groups at 24 h after HS is shown in Fig. 1C.

The myocardial HSP72 content in the 2W- and 8W-Sham and 2W-CAL rats was increased to approximately 20, 20, and 18-fold, respectively, of value for the control rats without HS treatment. However, no obvious induction of myocardial HSP72 was observed in the 8W-CAL (Fig. 1C).

Changes in HSF1 Contents and Subcellular Localization  Changes in the content of HSF1, transcription factor of HSPs, in the failing heart following MI are shown in Fig. 2. The cardiac HSF1 contents of the 2W- and 8W-CAL rats were increased to approximately 150% and 140%, respectively, of the corresponding values for the Sham rats (Fig. 2A). In the cytosolic fraction, the HSF1 content of the 2W-CAL rat was similar to that of the 2W-Sham. However, no obvious induction of myocardial HSF72 was observed in the 8W-CAL (Fig. 1C).
of that in the 8W-Sham rat heart (Fig. 2B). In the nuclear fraction before HS, the HSF1 content of 2W- and 8W-CAL rat was approximately 90% and 40%, respectively, of that of the corresponding Sham rats (Fig. 2C). The HSF1 content in the nuclear fraction of the 2W- and 8W-Sham and 2W-CAL rats at the end of exposure to HS was increased to approximately 22, 20, and 23-fold, respectively, of the value for the control rats without HS treatment. However, the HS-induced increase in HSF1 content in the nuclear fraction in the 8W-CAL animals was not seen (Fig. 2D).

The localization of myocardial HSF1 was examined immuno-histochemically (Fig. 3). Whereas HSF1 was present in the cytosol of the HS-untreated Sham and CAL hearts, obvious transfer of HSF1 from the cytosol into the nucleus was seen in the 2W- and 8W-Sham and 2W-CAL hearts after exposure to HS (Fig. 3). However, there was no significant increase in fluorescence of HSF1 in the nucleus of the failing (8W-CAL) heart after an exposure to HS (Fig. 3).

Furthermore, we measured the content of myocardial p-HSF1 (Ser303) of the 2W and 8W Sham and CAL rats, which is a cytosolic localization form of HSF1 (Fig. 4). The myocardial content of p-HSF1 in the 2W- and 8W-CAL rats

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**Fig. 3.** Immunohistochemical Analysis of Intracellular Location of HSF1 in the Sham-Operated (Sham) Rats and Coronary Artery-Ligated (CAL) Rats at the 2nd (2W) and 8th (8W) Weeks after Surgery

Whole-heart sections from 2nd and 8th weeks after surgery with or without heat shock were incubated with the HSF1 antibody, α-actinin antibody, and DAPI. HSF1 is identified by green fluorescence, and α-actinin positive cardiomyocytes are shown in red fluorescence. DAPI is identified by blue fluorescence. Scale bar represents 50µm.
was increased to approximately 170 and 300%, respectively, of that for the corresponding Sham rats (Fig. 4A). The p-HSF1 (Ser303) content in the cytosolic fraction from the 8W-CA l rats was increased to approximately 450% of that in this fraction from the 8W-Sham rats (Fig. 4B).

**Changes in Akt and GSK3β Contents** Changes in Akt and GSK3β contents of the viable LV from CA l rats and the LV from Sham rats at the 2nd and 8th week after the operation are shown in Fig. 5. The myocardial Akt content of the 2W- and 8W-CA l rats was approximately 150 and 90%, respectively, of that of the corresponding Sham rats (Figs. 5A, B). The myocardial content of Akt phosphorylated at its Ser473 in the 2W- and 8W-CA l rats was approximately 120 and 60% of that of the corresponding Sham rats, respectively (Figs. 5A, C). In the case of myocardial GSK3β, its content in the 2W- and 8W-CA l rats was similar to that in the corresponding Sham rats (Figs. 5E, F). The content of myocardial GSK3β phosphorylated at its Ser9 in the 2W- and 8W-CA l rats was approximately 90 and 50%, respectively, of that of the corresponding Sham rats (Fig. 5D). Thus, the ratio of the phosphorylated form of GSK3β to its non-phosphorylated one was significantly decreased in the 8W-CA l rats compared with that for the 8W-Sham ones (Fig. 5H).

**DISCUSSION**

In the present study, we observed a diastolic dysfunction, as suggested by increases in the E/A ratio and LVEDP at the 2nd week after the surgery. Decreases in the FS, EF, LVSP, and LV+dp/dt values also showed a systolic dysfunction in the 2W-CA l rat. In contrast, the cardiac pump function as indicated by CO index and SV index values were preserved in the 2W-CA l rats, suggesting that the cardiac function in these rats was preserved under the present experimental conditions. In the 8W-CA l rat, both diastolic and systolic functions were further decreased, and the CO index and SV index values were also decreased. These findings showed possible signs of chronic heart failure in this model, and are consistent with those in our previous study.16)

Recently, it is considered that heart failure is a chronic state of mechanical and metabolic stresses to the myocardium. Although, an increase in HSP72 would be beneficial against stress-induced tissue damage, we found that the HSP72 content in the failing heart was similar to that of the control animal without HS. In an earlier study, we obtained the first evidence that the induction of HSP72 following heat shock is blunted in the failing heart.4) In this present study, we measured the HSP72 content of Sham and CA l animals before heat shock treatment. This content in the 2W-CA l rats was slightly but significantly increased compared with that of the corresponding Sham rats. In contrast, the HSP72 content in the failing (8W-CA l) heart was similar to that in the heart of the 8W-Sham rats (Fig. 1A). Examination of the ability for HSP72 induction in the hearts following myocardial infarction under our experimental conditions revealed that the myocardial HSP72 content of the 2W- and 8W-Sham and 2W-CA l 24 h after exposure to HS was markedly increased. However, no obvious induction of HSP72 was seen in the 8W-CA l rats exposed to HS (Fig. 1C). These findings suggest that the induction ability of the failing heart to induce HSP72 after exposure to heat shock was blunted.

HSP72 transcription is predominantly regulated by HSF1.10,11) We examined the subcellular localization of HSF1 in the LV of Sham and the viable LV of CA l animals. Before the exposure to heat shock, the HSF1 content of the cytosolic fraction from the viable LV of 8W-CA l rat was increased and that in the nuclear fraction was simultaneously decreased compared with the 8W-Sham rats (Figs. 2B, C). After heat-shock treatment, the HSF1 content in the nuclear fraction of the 2W- and 8W-Sham and 2W-CA l was markedly increased to approximately 22, 20, and 23-fold, respectively, of that for the control rats without HS treatment. However, the HS-induced increase in the HSF1 content in the nuclear fraction from the failing (8W-CA l) heart was attenuated (Fig. 2D), in agreement with the results obtained by the immunohistochemical observations (Fig. 3). HSF1 is an important tran-
cription factor that is under strict posttranslational regulation. Despite the accumulating evidence for HSF1 in a multistep activation/inactivation cycle, the regulatory functions of posttranslationally modified HSF1 have yet remained to be understood. Recently, several studies have provided evidence for several phosphorylation sites in HSF1. It is assumed that their phosphorylation is necessary to exert primary the function of the transcription factor after heat shock exposure. It is well recognized that the phosphorylation of Ser303 in HSF1 inhibits the activation of HSF1. Substitution of this serine residue with alanine, such as in the HSF1 S303A mutant, results in increased transcriptional activity. Therefore, we determined the myocardial p-HSF1 (Ser303) content at the 2nd (2W) and 8th (8W) Weeks after surgery. We found that the myocardial p-HSF1 (Ser303) content in the 2W-CA1 rats was increased and further increased in the 8W-CA1 animals. The p-HSF1 (Ser303) content in the cytosolic fraction of the viable LV in the 8W-CA1 animals, but not in the 2W-CA1 ones, was markedly

![Graphs and images showing changes in Akt and GSK3β contents in the myocardium of Sham and CA1 animals at 2-week and 8-week intervals after surgery.](image-url)

**Fig. 5. Changes in Akt and GSK3β Contents of the Left Ventricle in the Sham-Operated (Sham) Rats and Coronary Artery-Ligated (CA1) Rats at the 2nd (2W) and 8th (8W) Weeks after Surgery**

(A–D) Changes in Akt and its phosphorylated form in the left ventricle of the Sham and CA1 animals. (A) Bands corresponding to Akt and p-Akt. (B) Cardiac Akt contents in the Sham and CA1 animals. (C) Cardiac p-Akt contents in the Sham and CA1 animals. (D) Ratio of p-Akt to Akt. (E–H) Changes in GSK3β and its phosphorylated form in the left ventricle of the Sham and CA1 animals. (E) Bands corresponding to GSK3β and p-GSK3β. (F) Cardiac GSK3β contents in the Sham and CA1 animals. (G) Cardiac p-GSK3β contents in the Sham and CA1 animals. (H) Ratio of p-GSK3β to GSK3β. Each value represents the mean±S.E.M. of 6 independent experiments. *p<0.05 vs. the corresponding Sham group. #p<0.05 vs. 2W CA1 group.
increased. These findings suggest that the activation of HSF1 was impaired via the phosphorylation of the Ser303 of HSF1 in the failing heart.

It is known that the Ser303 of HSF1 is phosphorylated by GSK3β and that the activity of GSK3β is reduced when this kinase is phosphorylated by protein kinase B (Akt). Therefore, we measured the contents of Akt, GSKβ, and their phosphorylation forms in the animals at the 2nd and 8th weeks after the surgery. The ratios of p-Akt/Akt and p-GSKβ/GSKβ in the failing heart were decreased compared with those for the 8W-Sham animals (Figs. 5D, H). These results suggest that the reduced levels of phosphorylated GSK3β (inactive) in the failing heart led to an increase in the amount of the active GSK3β enzyme for the phosphorylation of Ser303.

In conclusion, we have shown a possible mechanism underlying the impaired ability for induction of HSP72 in the failing heart. The impaired ability of the failing heart to induce HSP72 was at least in part mediated by the phosphorylation of the Ser303 residue of HSF1, repressing HSF1 function as a transcription factor. In the failing heart, an increase in HSP72 would be beneficial for reducing the damage to the myocardium.

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