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Effect of long-term treatment with trandolapril on Hsp72 and Hsp73 induction of the failing heart following myocardial infarction

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1 The effect of long-term treatment of rats with chronic heart failure (CHF) following acute myocardial infarction with trandolapril, an angiotensin I-converting enzyme (ACE) inhibitor, on heat shock-induced Hsp72 and Hsp73 production was examined.

2 Acute myocardial infarction was induced by coronary artery ligation (CAL). The animals with CAL showed symptoms of CHF at the 8th week after the operation. The hearts isolated from animals with CAL at the 2nd and 8th week after surgery were subjected to hyperthermia at 42°C for 15 min followed by 6-h perfusion (hyperthermia/6-h perfusion).

3 In the hearts isolated from the animals at the 2nd week, an approximate 20% decline in the rate pressure product (RPP) was seen after hyperthermia/6-h perfusion, which was similar to that in non-operated controls. In contrast, a significant reduction in the RPP after hyperthermia/6-h perfusion was seen in the hearts of rats with CHF. These hearts did not increase Hsp72 and Hsp73 production after hyperthermia. The decline in RPP was associated with failure in the production of myocardial Hsp72 and Hsp73.

4 When rats with CAL were treated with 3 mg kg^{-1} day^{-1} trandolapril from the 2nd to 8th week after the operation, the decline in RPP of the failing heart after hyperthermia was similar to that of the sham-operated rats. The induction of myocardial Hsp72 and Hsp73 production of the coronary artery-ligated rats after hyperthermia was reversed by treatment with trandolapril.

5 These findings suggest that the preserved ability to induce Hsp72 and Hsp73 production in the heart with CAL by trandolapril treatment may be attributed to the increased tolerance against heat stress-induced deterioration of myocardial contractile function.

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Abbreviations: ACE, angiotensin-I converting enzyme; ANOVA, analysis of variance; CAL, coronary artery ligation; CHF, chronic heart failure; Hsp, heat shock protein; HR, heart rate; KHM, Krebs-Henseleit-M199 medium; LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure; LVSP, left ventricular systolic pressure; MAP, mean arterial pressure; RPP, rate pressure product; SDS, sodium dodecylsulphate; TTC, triphenyltetrazolium chloride

Introduction

Heat shock protein 72 and 73 (Hsp72 and Hsp73) are inducible and constitutive components of normal cells and function as molecular chaperons in protein folding (Welch, 1987; Gething & Sambrook, 1992; Brown *et al*., 1993; Knowlton, 1995). There is increasing evidence for cardioprotection of Hsp72 against various stresses in cardiomyocytes, normal hearts, normal animals, or transgenic animals (Marber *et al*., 1993; 1995; Hutter *et al*., 1994; 1996; Plumier *et al*., 1995; Nakano *et al*., 1997; Brar *et al*., 1999; Kawana *et al*., 2000). A possible involvement of Hsp72 in the protective function has also been proposed in animals in the pathophysiological condition (Ferrari *et al*., 1996; Tanonaka *et al*., 2001). In a previous study, we observed that the failing heart following acute myocardial infarction did not enhance the production of Hsp72 and Hsp73 after an exposure to heat stress, and that there was a close relationship between the heat stress-induced decrease in contractile function of the perfused heart and attenuation of Hsp72 or Hsp73 production (Tanonaka *et al*., 2001). These findings suggest that the failure in production of these two proteins may enhance stress-induced cardiac dysfunction of the failing heart.

Numerous studies have shown the benefits of ACE inhibitors to the treatment of patients with cardiac failure (SOLVD Investigators, 1991; SAVE Investigators, 1992; TRACE Study Group, 1995). We showed in previous studies that treatment of rats with chronic heart failure (CHF) following myocardial infarction with trandolapril, an ACE inhibitor, elicited restoration of cardiac energy metabolism (Sanbe *et al*., 1995) and myocardial β-adrenoceptor density (Sanbe & Takeo, 1995), recovery of haemodynamic parameters (Sanbe *et al*., 1995), and improvement of exercise

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capacity of the skeletal muscle (Yamaguchi *et al*., 1999). However, no information is available concerning the effects of ACE inhibitors on stress-induced production of Hsp72 and Hsp73 in the failing heart. In the present study, we examined the effect of trandolapril treatment on the production of Hsp72 and Hsp73 in the failing heart following myocardial infarction.

Methods

Animals

Male Wistar rats (SLC, Hamamatsu, Japan), weighing 210–240 g, were used in the present study. The animals were conditioned at $23\pm1^{\circ}$C with a constant humidity of $55\pm5\%$ and a cycle of 12-h light/12-h darkness, and given free access to foods and tap water according to The Guide for the Care and Use of Laboratory Animals as promulgated by the National Research Council. The protocol of this study was approved by the Committee of Animal Use and Welfare of Tokyo University of Pharmacy and Life Science.

Operation

Myocardial infarction of rats was produced by occlusion of the left ventricular coronary artery according to the method described previously (Sanbe *et al*., 1993). The animals were anaesthetized by an intraperitoneal injection of 40 mg kg^{-1} pentobarbitone and artificially ventilated with air. The left coronary artery was ligated at approximately 2 mm from its origin with a suture. In the present study, two elimination criteria for selection of experimental animals were employed. One is the absence of abnormal Q wave (more than 0.3 mV) in ECG (lead I) 2 weeks after CAL. The other is more than 10 g increase in body weight of CAL animals 2 weeks after the operation. Using these elimination criteria, CAL rats with approximately 40% infarct area in the left ventricle were constantly produced. Seventy rats had received the operation of CAL. Eighteen rats died within 24 h and six within 1 week after the operation. Among the remaining 45 CAL rats, six CAL rats were eliminated from the present study according to the criteria of changes in body weight. Accordingly, 39 animals were used for following studies. No rats died between the 2nd and 8th week after surgery. Thirty Sham-operated rats were treated in a similar manner except for that coronary artery ligation was not performed (Sham rats).

Treatment with trandolapril

Rats were treated with an oral administration of 3 mg kg^{-1} of trandolapril once daily, from the 2nd to the 8th week after the operation as described previously. Trandolapril suspended in 0.3 ml of 0.25% sodium carboxymethyl cellulose was administered into the stomach *via* a probe. The dose of trandolapril employed in the present study was similar to that used in previous studies (Sanbe & Takeo, 1995; Sanbe *et al*., 1995).

Measurement of the in vivo *haemodynamic parameters*

Two (2w-CAL, 2w-Sham) and eight weeks (8w-CAL, 8w-Sham) after the operation, CAL and Sham rats were anaesthetized with a gas mixture of nitrous oxide-oxygen (3 : 1) and 2.5% enflurane (Yamaguchi *et al*., 1999). Anaesthesia was continued with a gas mixture of nitrous oxide-oxygen containing 0.5% enflurane at a flow rate of 600 ml min^{-1} through a mask loosely placed over the nose. The animals were warmed by an electronic panel heater to maintain their rectal temperature at $36.5\pm0.5^{\circ}$C. In a preliminary study, we performed the blood gas analysis of the experimental animals using a blood gas analyzer (Model 248, Chiron Diagnostics, East Walpole, MA, U.S.A.). The initial values for PO_2, PCO_2 and pH were 102.8 ± 2.1, 36.4 ± 1.2 and 7.42 ± 0.04 mmHg ($n=5$), respectively. There were no significant changes in these parameters throughout the experiment. There was no significant difference in the blood gas values between Sham rats and rats with 2w- or 8w-CAL.

A microtip pressure transducer (SPC-320, Millar Instrument, Houston, TX, U.S.A.) was introduced into the left ventricle through the carotid artery to measure left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP) and left ventricular dP/dt (+LV dP/dt, −LV dP/dt) by means of a pressure transducer (AB621G, Nihonkohden, Tokyo, Japan) and a differentiator (ED601G, Nihonkohden), respectively ($n=10$ each). The mean arterial blood pressure (MAP) was measured by means of a cannula placed into the right femoral artery and connected to a pressure transducer (DX360, Nihonkohden). Heart rate (HR), triggered from microtip transducer, was measured using a heart rate counter (AP601G, Nihonkohden). After an equilibration of 5-min setting period, the parameters were recorded using a thermal pen recorder (RTA1200, Nihonkohden).

Perfusion of hearts

After measurement of the *in vivo* haemodynamic parameters of the rats at the 2nd and 8th weeks following CAL ($n=10$ each), the hearts were rapidly isolated and transferred to a Langendorff apparatus. The hearts were perfused at 37°C at a constant flow rate of 9.0 ml min^{-1} with Krebs-Henseleit bicarbonate buffer (KH solution) of the following composition (mM): NaCl 120, KCl 4.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.25, $NaHCO_3$ 25, and glucose 11. The perfusion buffer was equilibrated with a gas mixture of 95% O_2 and 5% CO_2 (pH 7.4). A latex balloon with an uninflated diameter of 3.7 mm, and connected to a pressure transducer (TP200, Nihonkohden) was inserted into the left ventricular cavity through the mitral opening, and secured with a ligature that included the left arterial remnants. A 5-mmHg of the initial LVEDP was loaded to the perfused heart. After equilibration for 20 min, the hearts were perfused for 15 min at 42°C (hyperthermia) or 37°C (normothermia). Thereafter, the perfusion was continued for 6 h at 37°C with a mixture (3 : 1) of the Krebs-Henseleit buffer and Medium 199, a culture medium for cardiomyocytes that contains amino acids, vitamins, nucleosides, and nucleotides (KHM buffer), to support protein synthesis after the hyperthermia, as described previously (Tanonaka *et al*., 2001). These procedures were named as 'hyperthermia/6-h perfusion' and 'normothermia/6-h perfusion', respectively. After the 6-h perfusion, cardiac function of the perfused heart was

determined. Non-operated rats following the same procedure as above served as controls.

Determination of infarct size

In another set of experiments, the hearts of rats with CAL or Sham rats from the 1st to 8th week after surgery were isolated and sectioned into seven (1 mm-thick) slices from the base of the heart to the apex in a plane parallel to the atrioventricular groove ($n=3$ each). The slices were stained at 37°C for 10 min with 1% triphenyltetrazolium chloride (TTC) in saline. After staining, TTC-unstained areas, that is, infarct areas were determined according to the planimetric method which was based on determination of the epi- and endo-circumference of the infarct myocardium (Sanbe *et al*., 1993).

Determination of Hsp72 and Hsp73 by Western blot analysis

After hyperthermia/6-h perfusion or normothermia/6-h perfusion, hearts were quickly removed from the Langendorff apparatus. Then the viable left ventricle including the septum was separated from the heart, frozen, and stored in a container cooled at −80°C until assayed for Hsp72 and Hsp73 contents.

Each frozen tissue was homogenized with the buffer of the following composition (mM): sucrose 320, disodium EDTA 0.1, phenylmethanesulphonyl fluoride (PMSF), 0.1, and Tris/HCl, 10 (pH 7.4). The homogenate was centrifuged at $12{,}000\times g$ for 60 min at 4°C. Heat shock proteins in the supernatant fluid were fractioned and detected as described previously (Kawana *et al*., 2000). The resultant supernatant fluid was applied to an 8% SDS polyacrylamide gel (SDS–PAGE, 9×9 cm) according to the method of Laemmli (1970) with the buffer of the following composition (mM): Tris/HCl 49.7, glycine 384, SDS 3.47, pH 9.3. The proteins were separated in the gel at a constant current of 30 mA/gel for 2 h. Proteins separated on the gel were transferred to a polyvinylidene difluoride (PVDF) membrane (Immobilon PVDF, Millipore Co., Bedford, MA, U.S.A.) at a constant current of 2 mA cm^{-2} for 1 h with a buffer of the following composition; 192 mM glycine, 25.1 mM Tris, 20% (v v^{-1}) methanol. After blocking the membrane with Block Ace^{R} (Dainippon Pharm. Co. Ltd., Osaka, Japan) for 1 h, the transferred Hsp72 and Hsp73 on the PVDF membrane were detected with an anti-Hsp72 (Calbiochem-Novabiochem Co., San Diego, CA, U.S.A.) or anti-Hsp73 antibody (Affinity BioReagents, Golden, CO, U.S.A.). Thereafter, the HSPs on the membrane were visualized using a chemiluminescent system (ECL™, Amersham Pharmacia Biotech, Buckinghamshire, U.K.). The bands of these proteins developed on the X-ray films were scanned using a densitometric scanner (ES2000, Epson, Tokyo, Japan). The content of the proteins was semi-quantified and evaluated with a Densitograph^{R} (Atto, Tokyo, Japan).

Statistics

The results were expressed as the means ± s.e.mean. Statistical significance was estimated by analysis of variance (ANOVA) followed by Fisher's multiple comparison. Differences with a probability of less than 5% were considered to be statistically significant ($P<0.05$).

Results

Haemodynamics of the CAL rat in vivo *and infarct size*

Haemodynamic parameters of CAL and Sham rats *in vivo* were measured 2 and 8 weeks after the operation (Table 1). The MAP, LVSP, and ± LV dP/dt of the CAL rats were decreased 2 and 8 weeks after surgery, whereas HR was not altered at these periods. In contrast, the LVEDP was increased 2 weeks after CAL, and then further increased 8 weeks. There were no changes in these haemodynamic parameters of the Sham rats throughout the experiment. In another set of experiments, the infarct areas of the 2w- and 8w-CAL rats covered approximately 40% of the left ventricle (Table 1). There was no infarction in the myocardium of the Sham rats.

Treatment of the rats with CAL with 3 mg kg^{-1} day^{-1} trandolapril during the 2nd to 8th week after the operation attenuated the increase in LVEDP. Positive and negative LV dP/dt of 8w-CAL rats treated with trandolapril tended to be higher than those treated without the agent. Treatment of rats with CHF and Sham rats with trandolapril showed decreased MAP and LVSP compared with the corresponding animals treated without the agent. Myocardial infarct size was not altered by treatment with the agent (Table 1).

Induction of Hsp72 and Hsp73 in control rat hearts

To elucidate the optimal conditions for induction of Hsp72 and Hsp73, we subjected the hearts isolated from non-operated rats (control rats) to 15-min hyperthermia at a temperature of 42°C, followed by different periods of normothermic perfusion ranging from 0 to 8 h. Figure 1 shows the time course of the expression of Hsp72 and Hsp73 detected by Western blotting analysis (upper panels in Figure 1) and their densitometric quantification (lower panels in Figure 1). The peak levels of both Hsp72 and Hsp73 in the perfused hearts were seen 6 h after hyperthermia. Thus, we used 6-h perfusion with KHM buffer following hyperthermia to induce the peak levels of both Hsp72 and Hsp73.

Production of myocardial Hsp72 and Hsp73 in CAL and Sham rats

Myocardial Hsp72 and Hsp73 contents of control, Sham and CAL rats were examined after the normothermia/6-h perfusion and hyperthermia/6-h perfusion (Figures 2 and 3; $n=5$ each). In the control hearts, the Hsp72 content of the left ventricles was 583 ± 27% and the Hsp73 content was 240 ± 9% of each value for the normothermic animal after the hyperthermia/6-h perfusion.

When the isolated hearts were subjected to 20-min normothermia followed by 6 h-normothermic perfusion, the Hsp72 content of 2w- and 8w-CAL rats was similar to that of the control or the corresponding Sham rats (Figure 2; $n=5$ each). When the hearts were exposed to hyperthermia and then perfused for 6 h under normothermic conditions, the Hsp72 content of 2w-CAL rats was approximately 450% of

Table 1 *In vivo* haemodynamic parameters of coronary artery-ligated and sham-operated rats at the 2nd and 8th weeks after the operation

			Time after coronary artery ligation (weeks)		
				8th	
	Group	*Cont*	*2nd*	*None*	*Trandolapril*
Heart rate	Sham	398±8	405±8	411±10	402±8
(beats min^{-})	CAL		391±11	397±12	390±9
MAP	Sham	122±3	125±7	116±8	105±5
(mmHg)	CAL		104±4*	102±5*	82±3*#
LVSP	Sham	133±2	136±5	131±6	115±4#
(mmHg)	CAL		112±5*	108±4*	104±3*
LVEDP	Sham	5.1±0.8	4.9±0.7	4.9±0.7	5.3±0.6
(mmHg)	CAL		21.4±1.6*	32.4±1.2*	19.1±1.8*#
Infarct size	Sham	N.D.	N.D.	N.D.	N.D.
(% LV)	CAL		43.9±0.8	42.8±0.5	43.1±0.6

Heart rate, mean arterial pressure (MAP), left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP) were determined in the rats at the 2nd and 8th weeks after coronary artery ligation or sham operation. In another set of experiments, the infarct size of rats with coronary artery ligation was determined ($n=3$ each). Values for heart rate, MAP, LVSP and LVEDP represent the means±s.e.mean of 10 (Cont and Sham groups) or 13 experiments (CAL groups). *Significantly different from the corresponding value for the sham-operated rats ($P<0.05$). #Significantly different from the CAL rats treated without the agent ($P<0.05$). Abbreviations: N.D., not detectable.

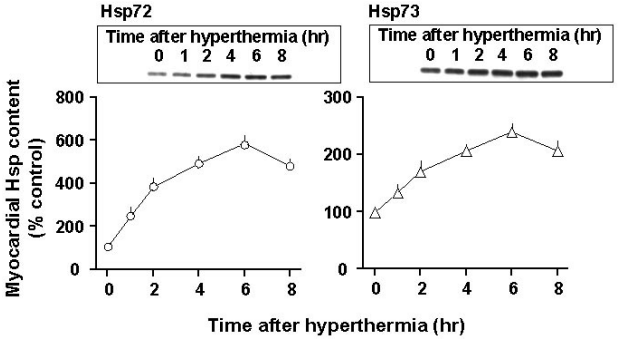

Figure 1 Western blots of Hsp72 (upper left panel) and Hsp73 (upper right panel) and the time course of changes in the production of Hsp72 (lower left panel) and Hsp73 (lower right panel) of the control rat hearts. The production was induced by hyperthermia followed by different perfusion periods ranging from 0 to 8 h. The production of these proteins is shown as the per cent change to control value for hearts without hyperthermia and 6-h perfusion. Each value represents the mean±s.e.mean of 3–5 experiments.

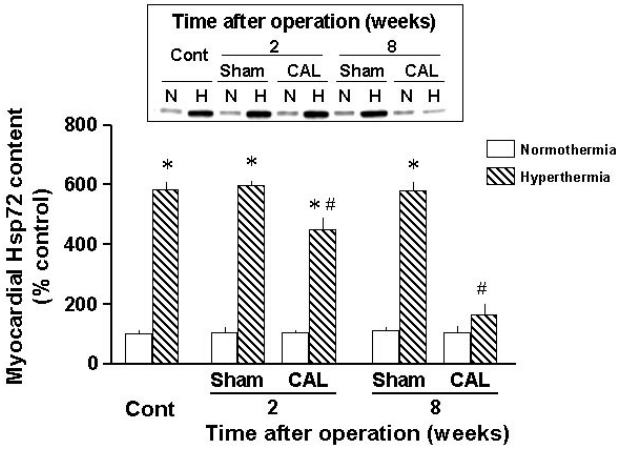

Figure 2 Hsp72 production of the viable left ventricle perfused for 6 h following 15 min normothermia (open columns) or hyperthermia (hatched columns). The hearts were isolated from the control (Cont), CAL, and Sham rats 2 and 8 weeks after the operation. In the upper panel, 'N' and 'H' mean normothermic and hyperthermic groups, respectively. Each value represents the mean±s.e.mean of five experiments. *Significantly different from the corresponding normothermic group ($P<0.05$). #Significantly different from the corresponding hearts from Sham rats subjected to hyperthermia ($P<0.05$).

the controls. In contrast, the Hsp72 content of 8w-CAL rats was 155% of the controls, whose increase was not significant compared with the control value.

The hearts of 2w-Sham or 2w-CAL rats showed that the increase in Hsp73 after the hyperthermia/6-h perfusion was similar to that of the control rats (Figure 3; $n=5$ each). In contrast, there were no significant increases in Hsp73 content of the 8w-CAL rats, regardless of the exposure to normothermia or hyperthermia.

Figures 4 and 5 show the effects of long-term treatment of Sham or CAL rats at the 8th week after the operation with trandolapril on the production of the Hsp72 and Hsp73 after hyperthermia/6-h perfusion. When the CAL rats were treated with trandolapril, Hsp72 and Hsp73 contents after hyperthermia/6-h perfusion was approximately 500 and 220% of each control, respectively. In Sham rat hearts, changes in the Hsp72 and Hsp73 contents in the left ventricles after the normothermia/6-h perfusion or hyperthermia/6-h perfusion were similar to those of the control rat hearts throughout the experiment. Treatment of Sham rats with trandolapril did not alter these two proteins after normothermia/6-h perfusion or hyperthermia/6-h perfusion.

Cardiac function of perfused hearts

Figure 6 shows LVDP, HR, and RPP of perfused hearts of CAL and control rats prior to hyperthermia, which represent baseline values for cardiac function of *in vitro* animals ($n=10$ each). The values for LVDP, HR, and RPP of the perfused hearts of control rats were 79.4±2.3 mmHg, 280±11 min^{-1}, and 22160±1200 mmHg*min^{-1}, respectively. Both LVDP and RPP of 2w-CAL rats decreased to approximately 82% of the 2w-Sham rats, whereas HR did not alter. The LVDP, HR, and RPP of 8w-CAL rats declined to approximately 62, 90, and 54% of the 8w-Sham rats. The LVDP, HR, and RPP

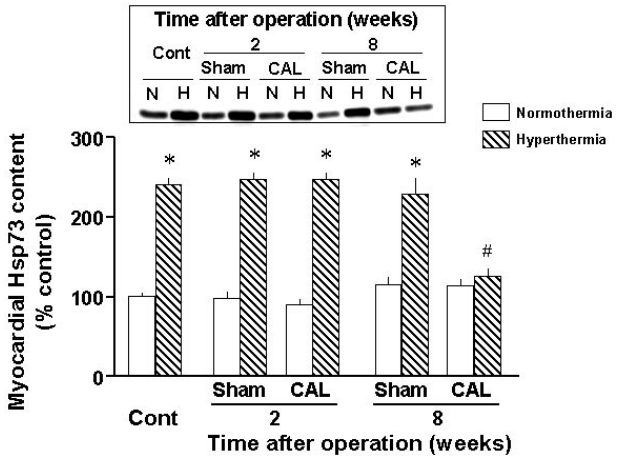

Figure 3 Hsp73 production of the viable left ventricle perfused for 6 h following 15 min normothermia (open columns) or hyperthermia (hatched columns). The hearts were isolated from the control (Cont), CAL, and Sham rats 2 and 8 weeks after the operation. In the upper panel, 'N' and 'H' mean normothermic and hyperthermic groups, respectively. Each value represents the mean ± s.e.mean of five experiments. *Significantly different from the corresponding normothermia group ($P<0.05$). #Significantly different from the corresponding hearts of Sham rats subjected to hyperthermia ($P<0.05$).

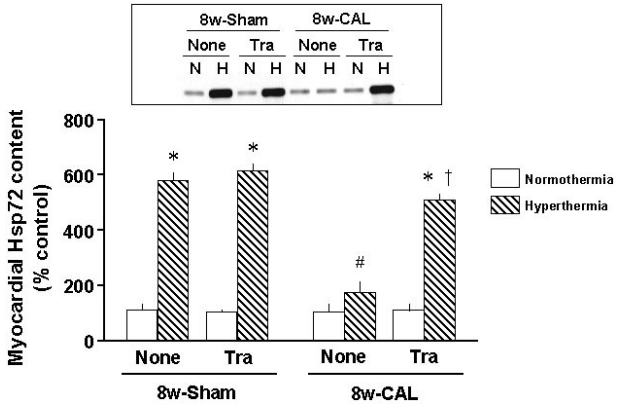

Figure 4 Western blot of Hsp72 (upper panel) and its production (lower panel) of the viable left ventricle perfused for 6 h following 15 min normothermia (open columns) or hyperthermia (hatched columns). The hearts were isolated from the CAL (8w-CAL) and Sham rats (8w-Sham) treated with (Tra) and without trandolapril (None) 8 weeks after the operation. In the upper panel, 'N' and 'H' mean normothermic and hyperthermic groups, respectively. Each value represents the mean ± s.e.mean of five experiments. *Significantly different from the corresponding normothermia group of Sham rats ($P<0.05$). †Significantly different from the corresponding None group ($P<0.05$). #Significantly different from the corresponding Sham group ($P<0.05$).

of the 8w-CAL, trandolapril-treated rats were similar to those of 2w-CAL rats. The values for these parameters of 2w- and 8w-Sham rats were similar to those of the controls. There were no differences in the RPPs of the 8w-Sham rats regardless of treatment with or without the agent.

Figure 7 shows decreases in the contractile parameters of the perfused hearts of control, Sham, and CAL rats ($n=5$ each). The per cent changes in the LVDP, HR, and RPP before and after normothermia or hyperthermia/6-h perfusion were calculated. The LVDP, HR, and RPP of the control

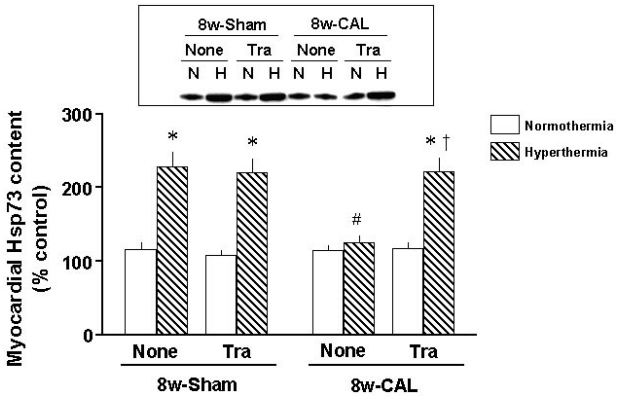

Figure 5 Western blot of Hsp73 (upper panel) and its production (lower panel) of the viable left ventricle perfused for 6 h following 15-min normothermia (open columns) or hyperthermia (hatched columns). The hearts were isolated from the CAL (8w-CAL) and Sham rats (8w-Sham) treated with (Tra) and without trandolapril (None) 8 weeks after the operation. In the upper panel, 'N' and 'H' mean normothermic and hyperthermic groups, respectively. Each value represents the mean ± s.e.mean of five experiments. *Significantly different from the corresponding normothermia group of Sham rats ($P<0.05$). †Significantly different from the corresponding None group ($P<0.05$). #Significantly different from the corresponding Sham group ($P<0.05$).

hearts gradually decreased to approximately 87, 95, and 83% of each initial value during the 6-h perfusion. When the control heart was subjected to the hyperthermia/6-h perfusion, a slight decrease in these parameters was also seen. Similar changes in these parameters were seen in the perfused heart of 2w- and 8w-Sham rats.

The LVDP, HR, and RPP of perfused hearts isolated from 2w-CAL rats gradually decreased to approximately 81, 97, and 80% of each initial value after the hyperthermia/6-h perfusion. There were no significant differences in the changes in these parameters between hyperthermic and normothermic hearts. In the hearts of 8w-CAL rats, the degrees of the changes in these parameters of the hearts with the normothermia/6-h perfusion were similar to those of the hearts isolated from 8w-Sham rats. In contrast, in the hearts of the CAL rats with hyperthermia/6-h perfusion, the decreases in the LVDP, HR, and RPP were significantly greater than that in the hearts isolated from the CAL animals with normothermia/6-h perfusion.

When the CAL rats were treated with trandolapril, the degrees of the decreases in the HR, LVDP, and RPP were similar to those of the controls. The changes in these parameters of the Sham rats treated with trandolapril during normothermia or hyperthermia/6-h perfusion were also similar to those of the controls.

Discussion

In the present study, we assessed at first the haemodynamic parameters of the animals prior to CAL, and 2 and 8 weeks after CAL to examine the pathogenesis of the operated animals. Decreases in MAP and LVSP, and an increase in LVEDP, were observed in the CAL animals compared with those in Sham rats. In previous studies, our laboratory reported that CAL rats showed a significant decrease in

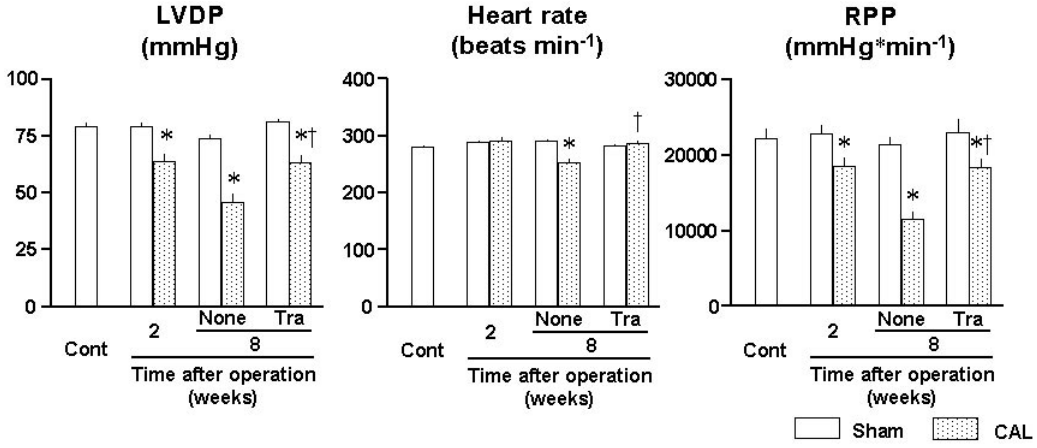

Figure 6 Baseline values for left ventricular developed pressure (LVDP, mmHg; the left panel), heart rate (HR, beats min^{-1}; the middle panel), and rate pressure product (RPP, $\times 10^3$ mmHg*min^{-1}; the right panel) of perfused hearts of control rats (Cont), the rats with coronary artery ligation treated with (Tra) and without trandolapril (None) 2 and 8 weeks after the operation. The open columns represent control or Sham rats. The hatched columns represent CAL rats. The hearts were isolated prior to coronary artery ligation (control) and one, two, and eight weeks after CAL. Each value represents the mean ± s.e.mean of five experiments. *Significantly different from the corresponding Sham value ($P<0.05$). †Significantly different from trandolapril-untreated value (None) at the 8th week after CAL ($P<0.05$).

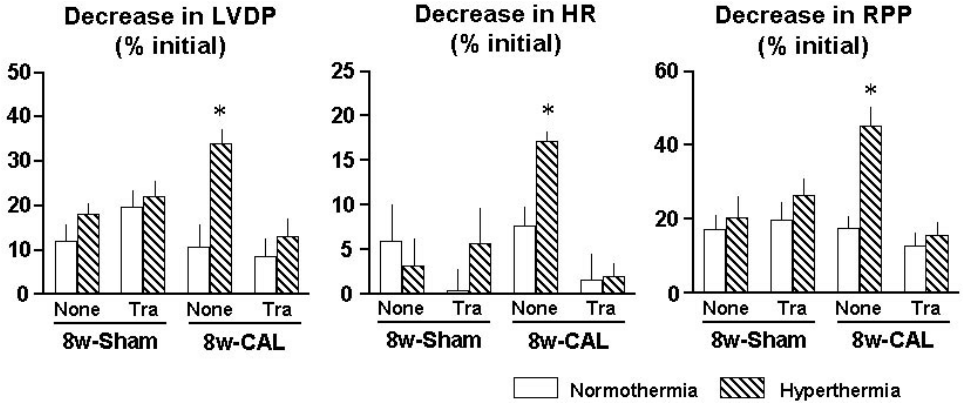

Figure 7 Decreases in left ventricular developed pressure (LVDP, the left panel), heart rate (HR, the middle panel), and rate pressure product (RPP, the right panel) of the hearts after 6 h perfusion following 15 min normothermia (open columns) or hyperthermia (hatched columns). The hearts were isolated from Sham (Sham) or CAL rats (CAL) treated with (Tra) and without trandolapril (None) 8 weeks after the operation. Each value represents the mean ± s.e.mean of five experiments. *Significantly different from the corresponding normothermia group ($P<0.05$).

LVSP without changes in cardiac output and stroke volume indices of the animals, at least up to the 4th week after CAL, whereas the latter parameters were decreased 8 weeks after CAL, which suggested the development of CHF (Sanbe *et al*., 1993; 1995). The findings in the present study were similar to those in the previous studies. Thus, it was suggested that 8w-CAL rats were in a failing stage following acute myocardial infarction, whereas 2w-CAL rats were in a compensatory stage. When the CAL rats were treated with trandolapril, the increase in LVEDP of CAL rats during the 2nd to 8th week was attenuated. These findings are similar to those of our previous studies (Sanbe *et al*., 1995; Yamaguchi *et al*., 1999), suggesting that treatment with the agent may improve the pathophysiological state of the rats with CHF.

After measurement of the *in vivo* haemodynamic parameters, the ability of the heart to produce Hsp72 and Hsp73 was determined using isolated, perfused preparations. This method is capable of directly estimating the myocardial protein production and cardiac function under conditions that can eliminate the effect of the systemic circulation. As shown in Figures 2 and 3, we found that Hsp72 and Hsp73 of the failing heart did not increase after an exposure to hyperthermia, whereas those of the hearts of 2w-CAL rats increased. When the CAL rats were treated with trandolapril for 6 weeks, the induction ability of Hsp72 recovered to approximately 85% of the Sham level and that of Hsp73, to the Sham level as shown in Figures 4 and 5. Treatment of the Sham or CAL rats with the agent did not alter Hsp72 nor Hsp73 of the hearts without an exposure to hyperthermia, suggesting that trandolapril itself does not directly induce myocardial Hsp72 or Hsp73. The present findings show that the development of CHF may attenuate or prevent the induction of both Hsp72 and Hsp73 in the CAL rat hearts and that long-term treatment of the CAL rats with trandolapril preserves the ability to produce these proteins.

For assessment of cardiac function, changes in the RPP of perfused hearts during the initial stage and after the hyperthermia/6 h perfusion were determined. The baseline

value for the RPP of 2w-CAL rats decreased and that of 8w-CAL rats further decreased, suggesting that the development of cardiac failure may deteriorate contractile function of the heart *per se*. When the CAL rats were treated with trandolapril, the decrease in the RPP of the isolated heart at the 8th week after CAL was attenuated compared with that of the untreated rats. These findings suggest that treatment with the agent may improve the contractile function of the heart with CAL.

As shown in Figure 7, a small decline in the RPP of control or Sham rats was observed after the hyperthermia/6-h perfusion. The per cent decrease in the RPP of the failing heart (8w-CAL group) after the hyperthermia/6-h perfusion was greater than those of any other group, suggesting that the failing heart reduces the tolerance against a hyperthermia-induced decrease in cardiac contractility. The degree of the decrease in RPP of the trandolapril-treated CAL rats after the hyperthermia/6-h perfusion was similar to that of the Sham rats. Therefore, treatment of CAL rats with trandolapril reversed the tolerance against the hyperthermia-induced decrease in cardiac contractility.

In a previous study, we observed the close relationship between Hsp72 or Hsp73 production and contractile function of perfused hearts after hyperthermia (Tanonaka *et al*., 2001). This finding shows that long-term treatment with trandolapril may improve Hsp72 and Hsp73 production of the hearts with CAL after an exposure to heat stress. This restoration may lead to an increase in tolerance of the heart against heat stress-induced cell damage.

Several studies have shown changes in myocardial Hsp72 content under pathophysiological conditions. For example, production of myocardial Hsp72 was enhanced in the young spontaneous hypertensive rat (Bongrazio *et al*., 1994; Gaia *et al*., 1995). A marked increase in the Hsp72 content of the right ventricle was observed in rats with monocrotaline-induced right heart failure (Comini *et al*., 1996). In contrast, the Hsp72 and Hsc73 contents of the human failing heart with ischaemic or dilated cardiomyopathy were similar to those of the human normal heart (Knowlton *et al*., 1998). We found that myocardial Hsp72 and Hsp73 of rats with CHF did not change under normothermic conditions compared with those of control and Sham rats. These findings showed that there were diverse induction patterns of Hsp72 under various pathological conditions. In a previous study, we found that hyperthermia did not induce Hsp72 or Hsp73 production of the failing heart (Tanonaka *et al*., 2001). Thus, it is likely that the failure of the induction of these proteins may be accompanied by functional deterioration of the failing hearts after hyperthermia.

Although there have been several studies concerning the involvement of Hsp72 in the pathological events of circulatory disease as described above, there is no information concerning therapeutic effects of agents on Hsp72 and Hsp73 production in failing hearts. The present study is the first to report the effects of an ACE inhibitor, one of the drugs used for heart failure, on the cellular defense mechanism against stress-induced myocardial injury of the failing heart. It remains unclear that recovery or preservation of the induction of these two proteins in the hearts of 8w-CAL rats is the cause or effect of treatment with trandolapril. The present findings suggest that myocardial Hsp72 and Hsp73 may be, at least in part, involved in the prevention of cardiac function of CHF animals.

At present, it is unclear what the roles of Hsp in the pathophysiology of experimental and clinical heart failure are. Therefore, the clinical implications of an altered response of the heart to transient exposure to heat stress are limited. In addition, it is impossible to estimate what could be the contribution of this effect of the observed benefit of ACE inhibitor after CAL. Further studies are necessary to explore the cellular defense mechanisms exerted by this agent.

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