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Effects of angiotensin I-converting enzyme inhibitor and angiotensin II type 1 receptor blocker on the right ventricular sarcoglycans and dystrophin after left coronary artery ligation

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Abstract

We examined the effects of trandolapril and candesartan on changes in the levels of sarcoglycans and dystrophin in the right ventricle of rats with the left coronary artery ligation. Hemodynamic and morphological alterations suggested the development of hypertrophy of the right ventricle and chronic heart failure by the 8th week. By the end of the 8th week, α- and β-sarcoglycans and dystrophin were decreased. Increases in μ- and m-calpains in the hypertrophied right ventricle were associated with an elevation of casein-proteolytic activity in the cytosolic fraction. Oral administration of 3 mg/kg/day trandolapril or 1 mg/kg/day candesartan from the 2nd to 8th week after the left coronary artery ligation attenuated decreases in α-sarcoglycan and dystrophin and reduced the increased proteolytic activity. The results suggest that attenuation of decreases in sarcoglycans and dystrophin is a possible mechanism underlying trandolapril- and candesartan-mediated improvement of structural and functional alterations of the right ventricle in the coronary artery-ligated rat.

Keywords: Angiotensin I-converting enzyme inhibitor; Angiotensin II type 1 receptor blocker; Calpain; Dystrophin glycoprotein complex; Hypertrophy; Myocardial infarction

1. Introduction

Sarcoglycans and dystrophin, the main components of the dystrophin glycoprotein complex, are located at the sarcolemma of cardiac, skeletal, and smooth muscle cells, and are believed to play a critical role in the stabilization of the sarcolemmal integrity (Ozawa et al., 1998). Genetically dystrophin glycoprotein complex-deficient humans and animals suffer severe muscular diseases. For example, an inherent mutation of the dystrophin gene causes Duchenne- or Becker-type muscular dystrophy and dilated cardiomyopathy (Feng et al., 2002; Hoogerwaard et al., 1999; Kunkel, 1986). Furthermore, limb-girdle muscular dystrophy (LGMD) is caused by the mutation of sarcoglycan subunits or by the deficiency of sarcoglycans, and thus several LGMDs are designated as the sarcoglycanopathy (Melacini et al., 1999). It has also been reported that degradation of the dystrophin glycoprotein complex, especially dystrophin, occurs in enterovirus-induced cardiomyopathy and in human ischemic cardiomyopathy (Badorff et al., 1999; Vatta et al., 2002).

Our previous studies have shown that the left coronary artery ligation of rats without genetic deficiency induced morphological alteration and degradation of dystrophin glycoprotein complex in the viable portion of the left ventricle (viable left ventricle), and thus suggested that degradation of those complexes contributes to the left ventricular dysfunction of failing hearts (Yoshida et al., 2003; Takahashi et al., 2005). This model revealed a decrease in cardiac output index, increases in left ventricular end-diastolic pressure and wet weight of the lung, dilatation of the left ventricle, and hypertrophy in the viable left ventricle (Sanbe et al., 1993, 1995; Yoshida et al., 2001b). Thus, the left ventricular muscle of coronary artery-ligated rats appears

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to be severely affected by the left ventricular failure and morphological alterations in the lungs. In contrast, the right ventricle of this model appeared to be indirectly influenced by the coronary artery ligation and impaired by the increased preload and afterload. In fact, we observed several differences in the morphological appearance between the right and left ventricles in previous studies (Sanbe et al., 1993, 1995). That is, a marked infarct area in the left ventricle, an extreme thinness of the left ventricular free wall, and hypertrophy of the viable left ventricle were seen, whereas hypertrophy of the right ventricle without any infarct area was observed. Such differences in the pathophysiological state between the right and left ventricles may provide diverse profiles for alteration of the dystrophin glycoprotein complex. In the present study, changes in degradation of the dystrophin glycoprotein complex and protein synthesis of the right ventricle in the failing heart following the left coronary artery ligation were examined.

Angiotensin I-converting enzyme inhibitor (ACE inhibitor) and angiotensin II type 1 (AT1) receptor blocker are well accepted as therapeutic agents for heart failure after acute myocardial infarction in humans and experimental animals (Capasso et al., 1994; Daniels et al., 2001; McKelvie et al., 1997; Pfeffer et al., 1987, 1992; Sweet et al., 1988). Long-term treatment of the coronary artery-ligated rat with ACE inhibitor or AT1 receptor blocker improved cardiac contractility, myocardial metabolism including energy-producing ability, signal transduction, and stress-induced expression of heat shock proteins (Sanbe et al., 1995; Tanonaka et al., 2001; Yoshida et al., 2001b). These effects are considered to be partly attributable to a reduced afterload and attenuation of cardiac remodeling such as suppression of collagen synthesis and hypertrophy (Sanbe et al., 1993, 1995; Yoshida et al., 2001a). The effects of ACE inhibitor and AT1 receptor blocker treatment on changes in the level of the dystrophin glycoprotein complex in the right ventricle of the failing heart, however, remain unclear. Therefore, as another part of the present study, the ACE inhibitor trandolapril or the AT1 receptor blocker candesartan was administered to coronary artery-ligated rats from the 2nd to 8th weeks and the effects of these agents on the level of the dystrophin glycoprotein complex in the right ventricle were examined.

2. Methods and materials

2.1. Animals and operation

Male Wistar rats (SLC, Hamamatsu, Japan), weighing 210–240 g, were used in the present study. The animals were conditioned according to Guide for the Care and Use of Laboratory Animals as published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The protocol of this study was approved by the Committee of Animal Use and Welfare of Tokyo University of Pharmacy and Life Science.

Myocardial infarction of rats was produced by ligation of the left ventricular coronary artery according to the method described previously (Sanbe et al., 1993). Sham-operated rats were treated in a similar manner except that no coronary artery ligation was done.

2.2. Treatment with drugs

Oral treatment of the coronary artery-ligated rats with 3 mg/kg/day of trandolapril (Aventis Pharma Japan, Tokyo, Japan) or 1 mg/kg/day of candesartan (Takeda Chem. Indust. Ltd., Osaka, Japan) once per day was performed by gavages from the 2nd to 8th week after the operation.

2.3. Measurement of hemodynamics and infarct size

At the end of the 2nd and 8th week, which are designated as "at the 2nd week" and "at the 8th week" in the following text, after coronary artery ligation or sham operation, hemodynamic parameters of anesthetized coronary artery-ligated and sham-operated rats at the 2nd and 8th weeks ($n=18$ each) were measured as described previously (Sanbe et al., 1993; Yoshida et al., 2001b). Briefly, coronary artery-ligated and sham-operated rats at the 2nd and 8th weeks were anesthetized with a gas mixture of nitrous oxide–oxygen (3 : 1) and 0.5–2.5% enflurane at a flow rate of 600 ml/min through a mask loosely placed over the nose. The pO_2, pCO_2, and pH of the blood were 95.2–109.8 mm Hg, 34.9–41.3 mm Hg, and 7.37–7.41, respectively. The left ventricular systolic pressure, left ventricular end-diastolic pressure, right ventricular systolic pressure, right ventricular end-diastolic pressure, mean arterial pressure, central venous pressure and heart rate were measured by means of pressure transducer (TP-200, Nihonkohden).

After determination of hemodynamic parameters of 18 rats in each group, 4 coronary artery-ligated and 4 sham-operated rats at the end of the 2nd or 8th weeks after the operation were used for determination of their infarct sizes by the planimetric method (Sanbe et al., 1993).

2.4. RT-PCR, and setting of experimental groups

The hearts of the other 14 rats in each group were quickly isolated and divided into the right ventricular free wall and the left ventricle including the infarct area and intermediate septum, and then their tissue weights were measured. Myocardial membrane and cytosolic fractions were prepared from the right ventricle of 5 coronary artery-ligated or sham-operated rats according to a modification of McMahon's method (McMahon, 1989). The membrane fraction was used for Western blot analysis of dystrophin glycoprotein complex, whereas the cytosolic fraction for determination of calpains and calpastatin. The hearts of 5 other rats in each group were used for reverse transcriptase polymerase chain reaction (RT-PCR) to determine mRNA expression of the dystrophin glycoprotein complex, and the last 4, for determination of the proteolytic activity in the right ventricle. The cDNAs were amplified by RT-PCR using the primers described in Table 1 (Hanada et al., 1997; Noguchi et al., 1999; Sandmann et al., 2001). Throughout the text, "control" refers to a non-operated, non-treated rat.

Table 1
Sense and antisense primers RT-PCR of α-, β-, γ-, δ-sarcoglycans (SG), dystrophin (Dys), and glyceraldehyde dehydrogenase (GAPDH)

Protein	Sequence		Reference
α-SG	Sense	ACTCACAGGGCTGGCTAGGCTGGAACA	Hanada
	Antisense	CGTCTGTCTGGTGCCGGAGGTGAAGAA	et al., 1997
β-SG	Sense	CAGGCTGCACCGGACCAAG	Hanada
	Antisense	AAGGTCAAGCTGAGATCGGATC	et al., 1997
γ-SG	Sense	TCGTCAGGAATCAGTTCCTCAGTG	Hanada
	Antisense	ACATGAAGGCTGAGGCACAGCTC	et al., 1997
δ-SG	Sense	CCATGACCACTGGATTCTCAAGG	Hanada
	Antisense	GATGGCTTCCATATTGCCAGCTTC	et al., 1997
Dystrophin	Sense	AACAACTGAACAGCCGGTGGACAG	Noguchi
	Antisense	TGACTGCTGGATCCACGTCCTGAT	et al., 1999
GAPDH	Sense	GAATTCCATTGACCTCAACTACATGG	Sandmann
	Antisense	TTGCTGCAGTCTTACTCCTTGGAGGCCAT	et al., 2001

2.5. Western blotting for sarcoglycans, dystrophin, calpain, and calpastatin

Western blotting analysis of α-, β-, γ-, and δ-sarcoglycans, dystrophin, μ- and m-calpains, and calpastatin was performed according to the method described previously with some modifications (Yoshida et al., 2003; Takahashi et al., 2005). Detection and quantification of these proteins on polyvinylidene fluoride membranes (Immobilon™-P, Millipore, Billerica, MA, USA) were performed by the method described earlier (Tanonaka et al., 2001).

2.6. Ex vivo proteolytic activity

In another set of experiments, the casein-proteolytic activity of the calpain-containing cytosolic fraction prepared from the right ventricular muscle of coronary artery-ligated rats at the 2nd and 8th weeks treated or not with a drug was estimated ($n=4$ each). The methods for preparation of the cytosolic fraction of the right ventricle and determination of leupeptin-sensitive casein-proteolytic activity were described previously (Yoshida et al., 2003).

2.7. Statistics

The results were expressed as means±S.E.M. Statistical significance was estimated by using two-way analysis of variance (ANOVA) followed by Fisher's PLSD correction for multiple comparisons, if necessary. Statistical significance between 2 parameters was calculated by use of Student's *t*-test. Differences with a probability of 5% or less were considered to be significant ($p<0.05$).

3. Results

3.1. Changes in hemodynamics and tissue weight

At the 2nd and 8th weeks after the left coronary artery ligation or sham operation, hemodynamic parameters of the animals were determined (Table 2). The right ventricular systolic pressure of the coronary artery-ligated rats at the 2nd week increased to approximately 2.4-fold the corresponding value for Sham animals. The right ventricular systolic pressure of the coronary artery-ligated rat at the 8th week further increased to 2.7-fold the value for sham-operated animal,

Table 2
Hemodynamic parameters of control (Cont), sham-operated (Sham) and coronary artery-ligated (CAL) rats treated or not (Non) with 3 mg/kg/day trandolapril (Tra) or 1 mg/kg/day candesartan (Can)

	MAP (mmHg)	CVP (mmHg)	HR (bpm)	RVSP (mmHg)	RVEDP (mmHg)	LVSP (mmHg)	LVEDP (mmHg)
Cont	114±3	2.5±0.6	405±2	27±2	1.1±0.2	149±3	1.6±0.3
2w-Sham	115±2	2.7±0.4	406±4	26±1	1.3±0.2	152±3	1.8±0.4
2w-CAL	105±2	8.2±0.9[a]	396±3	63±4	1.8±0.3	138±2[a]	20.5±1.2[a]
8w-Sham							
Non	118±2	2.7±0.5	402±2	25±2	1.1±0.1	154±4	1.3±0.4
Tra	100±3[b]	2.2±0.3	399±2	31±2	0.9±0.2	133±2[b]	1.1±0.3
Can	107±2[b]	2.1±0.3	404±3	29±2	1.0±0.2	145±2[b]	1.1±0.2
8w-CAL							
Non	108±2[a]	13.7±0.6[a]	397±3	68±4[a]	1.7±0.4	142±3[a]	32.3±1.1[a]
Tra	97±2[b]	5.1±0.5[a,b]	398±3	42±4[a,b]	0.8±0.2	111±3[a,b]	19.2±0.8[a,b]
Can	100±3[b]	5.8±0.7[a,b]	399±2	45±3[a,b]	1.0±0.1	121±3[a,b]	21.1±1.2[a,b]

Each value for hemodynamic parameters represents the mean±S.E.M. of 19 experiments. [a]$p<0.05$ vs. the corresponding Sham. [b]$p<0.05$ vs. the corresponding Non. Abbreviations: Cont, control rat; 2w- or 8w-Sham, sham-operated rats at the 2nd or 8th week after surgery; 2w- or 8w-CAL, coronary artery-ligated rats at the 2nd or 8th week after surgery; Non, non-treated animal with any drug; Tra, trandolapril-treated animals; Can, candesartan-treated animals; MAP, mean arterial pressure; CVP, central venous pressure; HR, heart rate; RVSP, right ventricular systolic pressure; RVEDP, right ventricular end-diastolic pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure.

whereas the right ventricular end-diastolic pressure of the coronary artery-ligated rats at the 2nd and 8th weeks was not altered. A rise in the blood pressure of the superior vena cava of the coronary artery-ligated rat was seen either at the 2nd or 8th week. There were no changes in these hemodynamic parameters of the sham-operated rat throughout the experiment when those parameters were compared with those of the control rat.

Treatment of coronary artery-ligated rats with trandolapril or candesartan from the 2nd to 8th week after the operation attenuated the increases in the right ventricular systolic pressure, central venous pressure, and left ventricular end-diastolic pressure of the coronary artery-ligated rat at the 8th week. The right ventricular end-diastolic pressures of coronary artery-ligated and sham-operated animals were not affected by treatment with either drug. Other hemodynamic parameters of coronary artery-ligated and sham-operated rats treated or not with drugs were similar to those in our studies reported previously (Sanbe et al., 1995; Takahashi et al., 2005; Tanonaka et al., 2001; Yoshida et al., 2001b).

Body, heart, and lung weights of the coronary artery-ligated and sham-operated rats at the 2nd and 8th weeks are shown in Table 3. The body weight of trandolapril- or candesartan-treated, sham-operated rats at the 8th week was significantly lower than that of the corresponding non-treated rat, whereas that of the drug-non-treated, coronary artery-ligated rats did not differ from that of the trandolapril- or candesartan-treated coronary artery-ligated rats. The right ventricular weight-to-body weight ratio for both coronary artery-ligated rats at the 2nd and 8th weeks increased as compared with that for the corresponding sham-operated rats. Long-term treatment with trandolapril or candesartan significantly attenuated the increases in the right ventricular weight-to-body weight ratio of the coronary artery-ligated rats at the 8th week. Other parameters for tissue weights of coronary artery-ligated and sham-operated rats treated or not with drugs were similar to those in our studies reported previously (Sanbe et al., 1995; Takahashi et al., 2005; Tanonaka et al., 2001; Yoshida et al., 2001b).

In another set of experiments, the infarct areas of the coronary artery-ligated rats were determined. The infarct areas of the coronary artery-ligated rats at the 2nd and 8th weeks covered approximately 40% of the left ventricle ($n=4$ each). Treatment with either drug had no effect on the infarct size of coronary artery-ligated rats (Table 3). No infarction was detected in the hearts of the control and sham-operated rats.

3.2. Myocardial dystrophin glycoprotein complex

Fig. 1 shows the changes in myocardial sarcoglycans and dystrophin contents of the right ventricle in sham-operated or coronary artery-ligated rats at the 2nd and 8th weeks treated or not with trandolapril or candesartan. α- and β-sarcoglycans in the right ventricle of the coronary artery-ligated rat at the 2nd week decreased to approximately 70% and 65% of the Sham value, respectively. At the 8th week after the left coronary artery ligation, α- and β-sarcoglycans and dystrophin in the right ventricle decreased to approximately 60%, 70%, and 70% of the value for the sham-operated rat, respectively. Treatment of coronary artery-ligated rats with trandolapril or candesartan restored α-sarcoglycan and dystrophin of the right ventricle to the control levels at the 8th week, while β-sarcoglycan tended to be reversed to the level of the sham-operated rat (to approximately 80% of the Sham value). γ- and δ-sarcoglycans in the right ventricle of the coronary artery-ligated rat did not change throughout the experiment, regardless of treatment or not with these drugs. The myocardial dystrophin glycoprotein complex contents in the sham-operated rat were similar to those of the control throughout the experiment regardless of treatment or not with drugs.

3.3. Myocardial calpains and calpastatin

Fig. 2 shows the changes in μ- and m-calpains and calpastatin in the right ventricle of the coronary artery-ligated

Table 3
Tissue weight and infarct size of control (Cont), sham-operated (Sham) and coronary artery-ligated (CAL) rats treated or not (Non) with 3 mg/kg/day trandolapril (Tra) or 1 mg/kg/day candesartan (Can)

	Body weight (g)	RV weight (mg)	RV/BW (mg/g)	LV weight (mg)	LV/BW (mg/g)	Lung weight (mg)	Lung/BW (mg/g)	Infarct size (% of total LV)
Cont	223±4	119±6	0.53±0.02	27±2	2.09±0.04	0.88±0.03	3.94±0.29	N.D.
2w-Sham	251±4	135±3	0.54±0.02	26±1	2.08±0.06	0.93±0.03	3.71±0.24	N.D.
2w-CAL	233±5	215±14[a]	0.92±0.04[a]	63±4	2.22±0.04	2.16±15[a]	9.27±0.54[a]	40.6±1.8
8w-Sham								
Non	311±7	163±11	0.52±0.02	25±2	1.97±0.03	1.01±0.04	3.24±0.29	N.D.
Tra	290±6[b]	131±10[b]	0.46±0.03	31±2	1.74±0.04[b]	0.97±0.03[b]	3.34±0.11	N.D.
Can	296±3[b]	134±9[b]	0.45±0.03	29±2	1.76±0.09	0.95±0.04[b]	3.21±0.13	N.D.
8w-CAL								
Non	288±6[a]	297±10[a]	1.03±0.04[a]	68±4[a]	2.11±0.04[a]	3.01±0.22[a]	10.45±0.76[a]	41.7±1.4
Tra	279±3[a,b]	211±11[a,b]	0.75±0.04[a,b]	42±4[a,b]	1.86±0.03[a,b]	1.84±0.10[a,b]	6.59±0.39[a,b]	40.9±1.6
Can	287±3[a]	224±8[a,b]	0.78±0.05[a,b]	45±3[a,b]	1.91±0.04[a]	2.07±0.14[a,b]	7.21±0.44[a,b]	41.1±2.1

Each value for tissue weight except for infarct size represents the mean±S.E.M. of 15 experiments, whereas the number of experimental animals used for the infarct size was 4. [a]$p<0.05$ vs. the corresponding Sham. [b]$p<0.05$ vs. the corresponding Non. Abbreviations: Cont, control rat; 2w- or 8w-Sham, sham-operated rats at the 2nd or 8th week after surgery; 2w- or 8w-CAL, coronary artery-ligated rats at the 2nd or 8th week after surgery; Non, non-treated animal with any drug; Tra, trandolapril-treated animals; Can, candesartan-treated animals; N.D., non-detectable; BW, body weight; RV, right ventricle; LV, left ventricle.

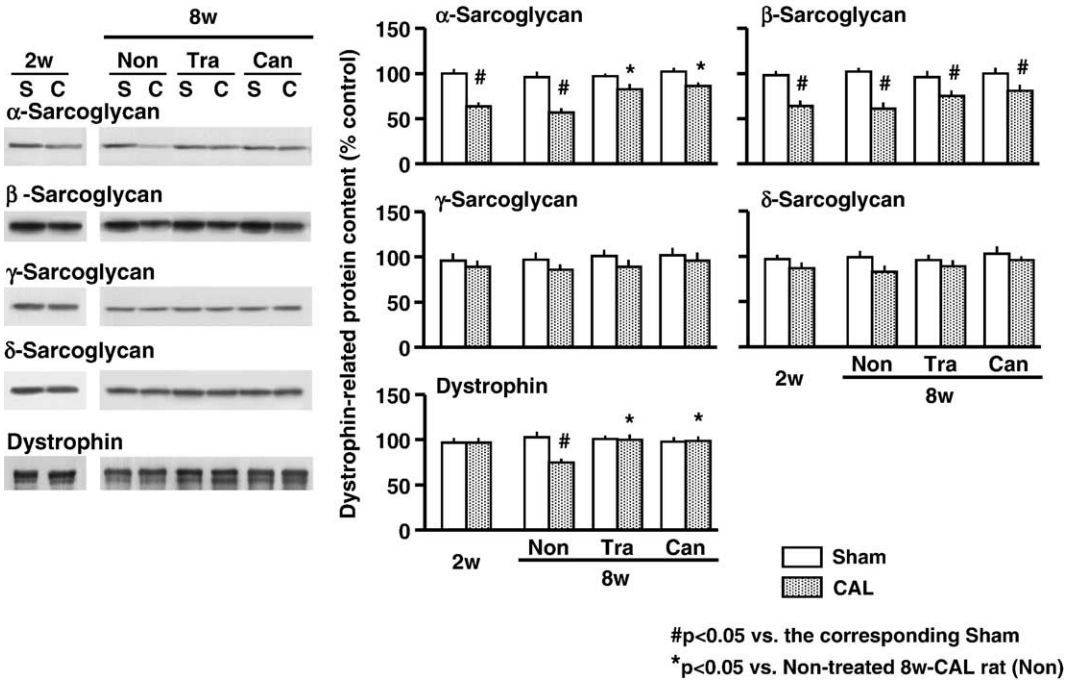

Fig. 1. α-, β-, γ-, and δ-sarcoglycans and dystrophin contents of the right ventricular tissue of sham-operated and coronary artery-ligated rats 2 or 8 weeks after the operation and the effects of treatment with trandolapril (Tra) and candesartan (Can) on these contents. Representative Western blots (left panel) indicate 50-kDa bands for α-sarcoglycan, 43-kDa for β-sarcoglycan, 35-kDa for γ-sarcoglycan, 35-kDa for δ-sarcoglycan, and 427-kDa for dystrophin in the right ventricle. "Non" indicates animals without drug treatment. Each value represents the mean±S.E.M. of 5 experiments. *$p<0.05$ vs. the corresponding sham-operated group. $^{\#}p<0.05$ vs. the corresponding "Non" group after the 8th week.

rats at the 2nd and 8th weeks treated or not with trandolapril or candesartan. Both μ- and m-calpain contents in the right ventricle of the coronary artery-ligated rats at the 2nd week increased to approximately 180% and 195% of the control, respectively. These calpains in the coronary artery-ligated rats at the 8th week also increased, and were similar to those of the coronary artery-ligated rat at the 2nd week (approximately 170% and 180% of the control, respectively). Treatment of the

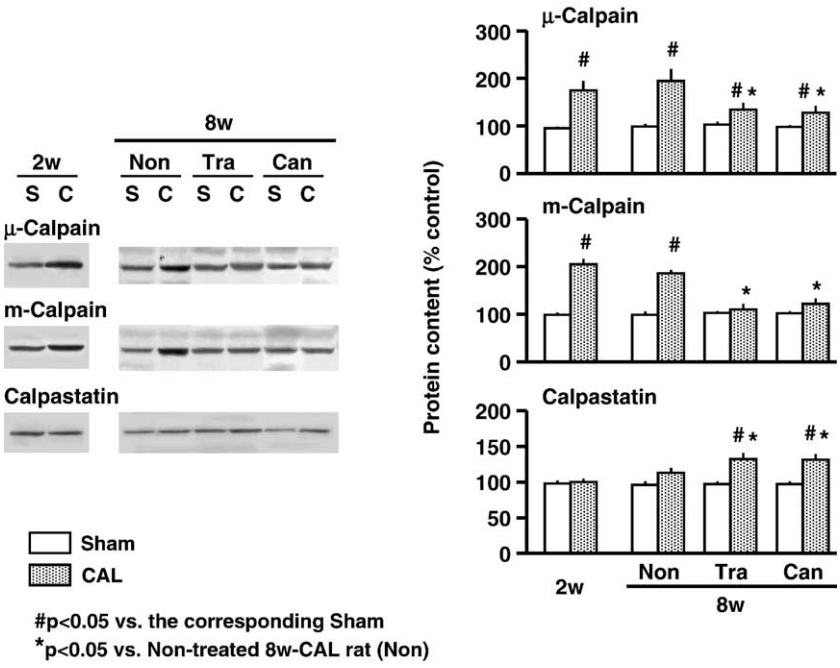

Fig. 2. μ- and m-calpains and calpastatin contents of the right ventricular tissue of sham-operated and coronary artery-ligated rats 2 or 8 weeks after the operation and the effects of treatment with trandolapril (Tra) and candesartan (Can) on these contents. Representative Western blots (left panel) indicate 80-kDa bands for μ-calpain, 80-kDa for m-calpain, and 70-kDa for calpastatin in the right ventricle. "Non" indicates animals without drug treatment. Each value represents the mean±S.E.M. of 5 experiments. *$p<0.05$ vs. the corresponding sham-operated group. $^{\#}p<0.05$ vs. the corresponding "Non" group after the 8th week.

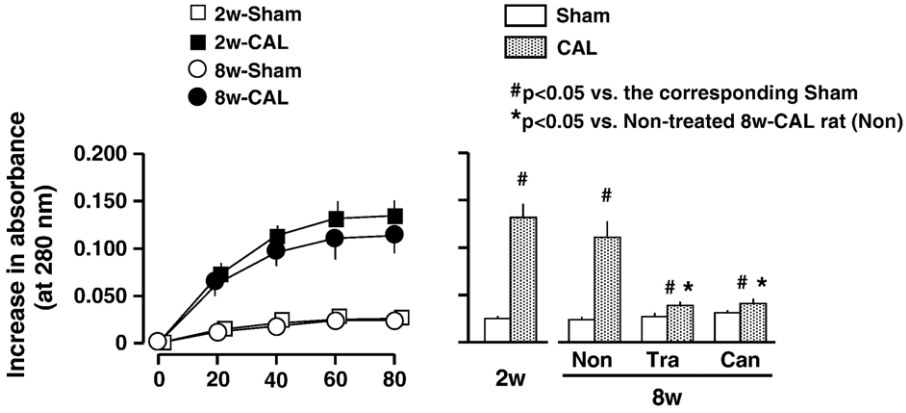

Fig. 3. Time course of changes in leupeptin-sensitive casein-proteolytic activity in the cytosolic fraction prepared from the right ventricular tissue of the sham-operated (open symbols) and coronary artery-ligated rats (closed symbols) in the presence of 5 mM $CaCl_2$ at the 2nd (squares) and 8th weeks (circles) after the operation (left panel). Leupeptin-sensitive casein-proteolytic activity of the cytosolic fractions prepared from the sham-operated (open columns) and coronary artery-ligated rats at the 8th week (hatched columns) without (Non) or with trandolapril (Tra) or candesartan (Can) treatment in the presence of 5 mM $CaCl_2$ is shown (right panel). Each value represents the mean±S.E.M. of 4 experiments. $^{\#}p<0.05$ vs. the corresponding sham-operated group. $*p<0.05$ vs. corresponding "Non" group after the 8th week.

coronary artery-ligated rats with trandolapril or candesartan significantly attenuated the increase in both calpains in the right ventricle of the coronary artery-ligated rats at the 8th week (approximately 125% and 110% of the control, respectively). There were no changes throughout the experiment in the μ- and m-calpains in the right ventricle of the sham-operated rats treated or not with trandolapril or candesartan.

Calpastatin of the right ventricle in the coronary artery-ligated rats at the 2nd and 8th weeks was similar to that of the corresponding Sham rats. Treatment of the coronary artery-ligated rats with trandolapril or candesartan increased calpastatin at the 8th week. There were no significant changes in calpastatin in the right ventricle of the sham-operated rats throughout the experiment regardless of treatment or not with drugs.

3.4. Calpain-like proteolytic activity of the cytosolic fraction

Next, the leupeptin-sensitive, Ca^{2+}-stimulated (calpain-like) proteolytic activity of the cytosolic fraction of the right ventricle from the coronary artery-ligated or sham-operated rat heart at the 2nd and 8th week was examined (Fig. 3). Casein was incubated in the presence of 5 mM $CaCl_2$ with the

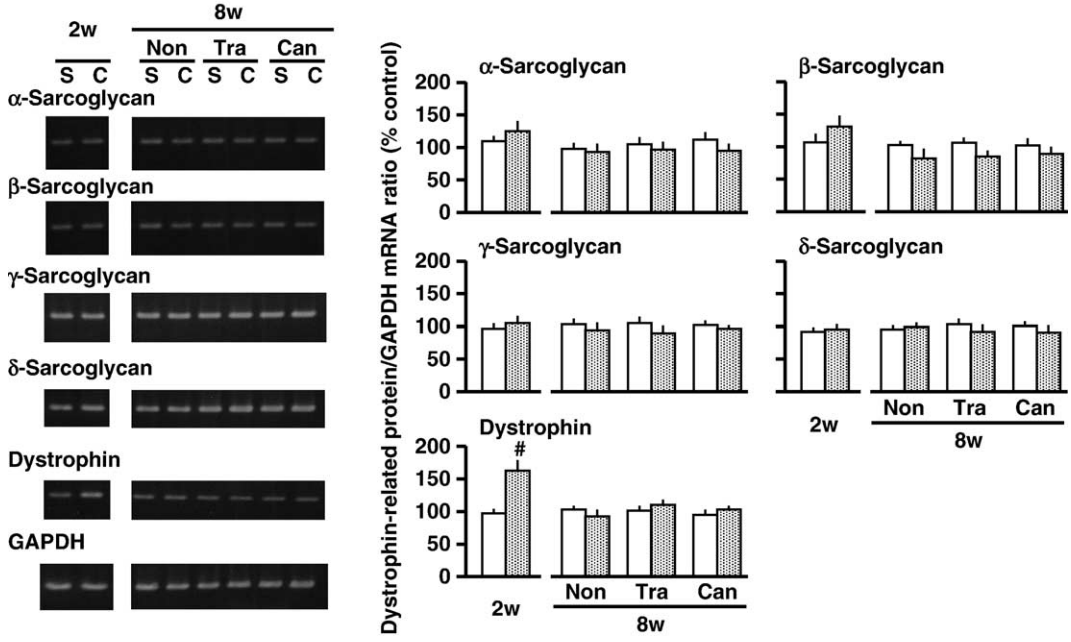

Fig. 4. Relative changes in mRNA expression levels of α-, β-, γ-, and δ-sarcoglycans and dystrophin in the right ventricular tissue of the sham-operated and coronary artery-ligated rats at the 2nd and 8th weeks and the effects of treatment with trandolapril (Tra) or candesartan (Can) on these mRNA levels. Left panels show representative PCRs that indicate mRNA levels of α-, β-, γ-, and δ-sarcoglycans, dystrophin, and GAPDH in the right ventricle. "Non" indicates animals without drug treatment. Each value represents the mean±S.E.M. of 5 experiments. $*p<0.05$ vs. the corresponding sham-operated group. $^{\#}p<0.05$ vs. the corresponding "Non" group after the 8th week.

cytosolic fraction prepared from the right ventricle of the coronary artery-ligated and sham-operated rats at the 2nd and 8th weeks. The left panel in Fig. 3 shows the time course of changes in the absorbance at 280 nm of the reaction mixture containing supernatant fluid prepared from the right ventricle of the coronary artery-ligated and sham-operated rats at the 2nd week, and the right panel, the activity for the coronary artery-ligated and sham-operated rats at the 8th week without and with trandolapril or candesartan treatment. In the presence of 5 mM $CaCl_2$, the absorbance reached its submaximal level after a 60-min incubation. As shown in the right panel of Fig. 3, treatment of the coronary artery-ligated rats with trandolapril or candesartan attenuated the increase in the casein-proteolytic activity in the cytosolic fraction of the coronary artery-ligated rat at the 8th week.

3.5. Transcriptional changes in dystrophin glycoprotein complex

Reverse transcription followed by PCR amplification of the total RNA resulted in a single band of the predicted size for myocardial sarcoglycans, dystrophin, or GAPDH. Fig. 4 shows the changes in mRNA levels of the right ventricular sarcoglycans and dystrophin of the coronary artery-ligated rats at the 2nd and 8th weeks treated or not with trandolapril or candesartan. mRNA levels of α- and β-sarcoglycans in the right ventricle of the coronary artery-ligated rat at the 2nd week tended to increase (approximately 125% and 120% of the control levels), whereas those of γ- and δ-sarcoglycans did not change. Dystrophin mRNA in the right ventricle of the coronary artery-ligated rat at the 2nd week increased (to approximately 150% of the control). The α-, γ-, and δ-sarcoglycans and dystrophin mRNA levels in the right ventricle of the coronary artery-ligated rat at the 8th week were similar to those of the corresponding sham-operated rat, whereas the mRNA level of β-sarcoglycan tended to be lower than that of the sham-operated rat (approximately 85% of the control). Treatment with trandolapril or candesartan did not affect the dystrophin glycoprotein complex mRNAs in the right ventricular of the coronary artery-ligated rat at the 8th week. The myocardial mRNA levels of the dystrophin glycoprotein complex in the sham-operated rats at the 2nd and 8th weeks were similar to those of the control rat, regardless of treatment or not with drugs.

4. Discussion

The present study showed that this model revealed increases in right ventricular systolic pressure, left ventricular end-diastolic pressure, and blood pressure of the superior vena cava and decreases in mean arterial pressure and left ventricular systolic pressure without changes in heart rate and right ventricular end-diastolic pressure at the 2nd and 8th weeks after the left coronary artery ligation. These hemodynamic profiles of the animal suggest the development of chronic heart failure by the 8th week (Sanbe et al., 1993, 1995; Tanonaka et al., 2001; Yoshida et al., 2003) and indicate increases in both preload and afterload to the right ventricle in coronary artery-ligated rats at the 2nd and 8th weeks after surgery. The morphological alterations including an increase in the right ventricle/body weight and an increase in the lung weight/body weight suggest the development of hypertrophy in the right ventricle and a marked change in the structure of the lung of the coronary artery-ligated rats at the 2nd and 8th weeks after the operation.

Under such a pathophysiological state, we observed that α- and β-sarcoglycans of the right ventricle of the coronary artery-ligated animal were decreased at the 2nd and 8th weeks, and dystrophin at the 8th week was reduced without changes in γ- and δ-sarcoglycans. The contents of μ- and m-calpain in the right ventricle of coronary artery-ligated animals were increased at the 2nd and 8th weeks after the operation without changes in calpastatin content, an intrinsic inhibitor for calpains. Furthermore, the cytosolic fraction prepared from the right ventricle of the coronary artery-ligated animals at the 2nd and 8th weeks showed an appreciable increase in the casein-proteolytic activity. These results suggest the possible involvement of calpain-mediated proteolysis in the degradation of dystrophin glycoprotein complexes in the coronary artery-ligated animal. Yoshida et al. (2003) reported that incubation of isolated sarcolemma with calpain in vitro resulted in rapid decreases in α- and β-sarcoglycans and a gradual decrease in dystrophin, suggesting different sensitivity of each component of dystrophin glycoprotein complexes to calpain-mediated proteolysis. We also showed that an elevation of the proteolytic activity in the cytosolic fraction of the coronary artery-ligated rats at the 2nd and 8th weeks was associated with increases in μ- and m-calpains. Furthermore, the increase in the calpains at the 2nd and 8th weeks was associated with decreases in α- and β-sarcoglycan contents of the right ventricle of the coronary artery-ligated rats at the 2nd and 8th weeks and in dystrophin in the right ventricle of the coronary artery-ligated rat at the 8th week. Thus, the activation of calpain is likely responsible for the degradation of dystrophin glycoprotein complex during the left coronary artery ligation-induced development of hypertrophy and/or heart failure.

We examined the relation between dystrophin glycoprotein complex content and its mRNA in the right ventricle of the coronary artery-ligated animal to elucidate the correlation between them, which relation is summarized in Table 4. After 2 weeks post coronary artery ligation, α- and β-sarcoglycan contents of the right ventricle were decreased without any change in their mRNA expression. At the 8th week, α-sarcoglycan, β-sarcoglycan, and dystrophin contents of the right ventricle were decreased without any change in their mRNA expression. There were no alterations in γ- and δ-sarcoglycan contents or their mRNA levels in the right ventricle throughout the experimental period examined. From these results we suggest that only some forms of dystrophin glycoprotein complex including α- and β-sarcoglycans and dystrophin are degraded during the development of cardiac hypertrophy and/or cardiac failure, unless de novo synthesis of the protein complex is enhanced to counteract the degradation.

Table 4
Summary of changes in levels of dystrophin-related proteins, calpain, and calpastatin in the viable LV and RV after coronary artery ligation and effects of trandolapril and candesartan

	Viable left ventricle (Takahashi et al., 2005)				Right ventricle			
	2nd week	8th week			2nd week	8th week		
		Non	Tra	Can		Non	Tra	Can
Protein content								
α-Sarcoglycan	±	↓	±	±	↓	↓	±	±
β-Sarcoglycan	±	±	±	±	↓	↓	↓	↓
γ-Sarcoglycan	±	±	±	±	±	±	±	±
δ-Sarcoglycan	±	±	±	±	±	±	±	±
Dystrophin	±	↓	±	±	±	↓	±	±
μ-Calpain	↑	↑	±	±	↑	↑	±	±
m-Calpain	↑	↑	±	±	↑	↑	±	±
Calpastatin	±	±	±	±	±	±	↑	↑
mRNA								
α-Sarcoglycan	↑	±	±	±	±	±	±	±
β-Sarcoglycan	↑	↑	±	±	±	±	±	±
γ-Sarcoglycan	±	±	±	±	±	±	±	±
δ-Sarcoglycan	±	±	±	±	±	±	±	±
Dystrophin	↑	±	±	±	↑	±	±	±
Proteolytic activity	↑	↑	±	±	↑	↑	±	±

Data on alterations in the above parameters of the left ventricle were derived from the previous study (Takahashi et al., 2005). "±", "↑", and "↓" in the table represent no change, increase, and decrease, as compared with the corresponding parameter of control animal, respectively.

Similar associated events were detected in a previous study in which we evaluated the alterations in dystrophin glycoprotein contents, calpain content, calpain-like activity, and mRNAs of the viable portion of the left ventricle in the coronary artery-ligated rats, as summarized in Table 4. That is, we observed no changes in α-sarcoglycan, β-sarcoglycan, and dystrophin contents with increases in these mRNAs at the 2nd week, no change in β-sarcoglycan content with an increase in β-sarcoglycan mRNA at the 8th week, and decreases in α-sarcoglycan and dystrophin contents without changes in α-sarcoglycan and dystrophin mRNAs at the 8th week after the left coronary artery ligation. Apparently, there was a persistent increase in the calpain content in the left ventricle of the coronary artery-ligated rats throughout the experiment. Thus, we hypothesized that degradation of the dystrophin glycoprotein complex occurs in the myocardial regions indirectly influenced by the left ventricular infarction in animals without any genetic mutation and that the degradation of dystrophin glycoprotein complex in animals with cardiac hypertrophy and/or cardiac failure is likely regulated by the activation of endogenous calpain (Fig. 5). The degradation appears to be also regulated by the balance between enhancement of degradation and synthesis of dystrophin glycoprotein complex.

Long-term treatment of the coronary artery-ligated rat with trandolapril or candesartan not only attenuated the coronary artery ligation-induced decrease in α-sarcoglycan but also completely reversed the left coronary artery ligation-induced decrease in dystrophin of the right ventricle of the coronary artery-ligated rats at the 8th week, showing that the effects of these drugs on α-sarcoglycan and dystrophin in the right ventricle are similar to those in the viable portion of the left ventricle in the coronary artery-ligated animal (Table 4). These drugs tended to recover the β-sarcoglycan of the right ventricle of coronary artery-ligated rats at the 8th week, but not significantly. These results indicate that ACE inhibitor and AT1 receptor blocker are capable of preventing the left coronary artery ligation-induced degradation of the dystrophin glycoprotein complex in the right ventricle that may be remotely affected by the left coronary artery ligation.

Treatment with trandolapril or candesartan attenuated the left coronary artery ligation-induced increases in μ- and m-calpains of the right ventricle of coronary artery-ligated rats at the 8th

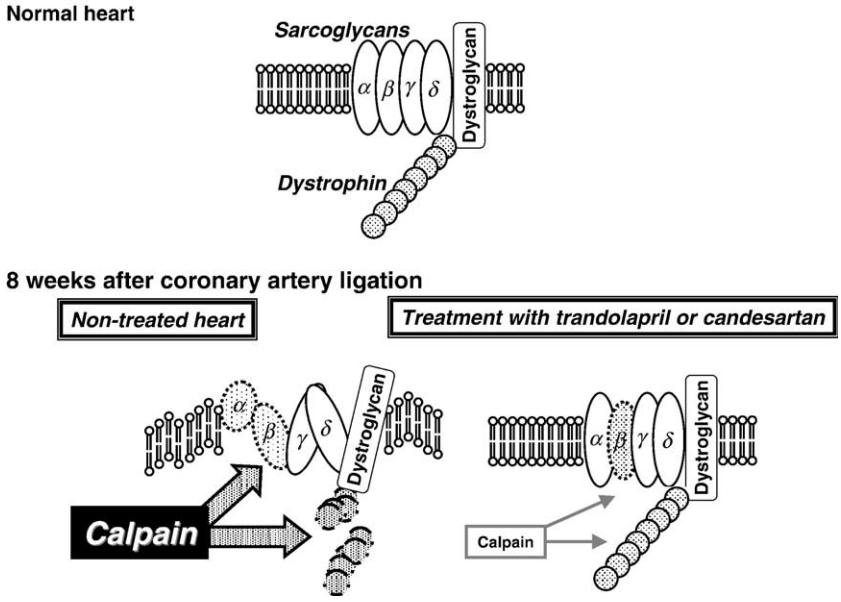

Fig. 5. Myocardial sarcoglycans and dystrophin protein at the 8th week after coronary artery ligation are digested by calpains, leading to degradation of dystrophin-related glycoprotein complex during the development of cardiac hypertrophy of the right ventricle. Long-term treatment with trandolapril and candesartan may preserve the protein complex and maintain cardiac contractility after coronary artery ligation.

week, whereas neither treatment altered the mRNA levels of sarcoglycans and dystrophin. Also, the treatment attenuated the increase in calpain-like proteolytic activity in the cytosolic fraction prepared from the coronary artery-ligated rat. In a preliminary study, we observed that the drugs did not directly inhibit in vitro casein proteolysis by calpain (data not shown). Therefore, it is likely that an attenuation of the increase in calpains as a result of reduced activation of calpain in the right ventricle of the drug-treated, coronary artery-ligated animal leads to prevention of the dystrophin glycoprotein complex proteolysis. In this sense, an increase in calpastatin, an intrinsic calpain inhibitor, in the drug-treated rat heart may contribute to the reduction in the proteolytic activity (Fig. 5).

Treatment with trandolapril or candesartan improved the hemodynamics of the right ventricle of the coronary artery-ligated rats at the 8th week, including the reductions in right ventricular systolic pressure and blood pressure of the superior vena cava. Although improvement of hemodynamics of drug-treated animals may be one of the possible mechanisms responsible for alteration of the degradation of dystrophin glycoprotein complex, it appears that the effects of the drugs on calpains provide a key for drug-mediated improvement of contractility in failing hearts.

Sandman et al. showed that ACE inhibitor or AT1 receptor blocker treatment of the coronary artery-ligated rat from 7 days before coronary artery ligation to 2 weeks after it attenuated increases in μ- and m-caplains in the non-infarct myocardium at the 2nd week after coronary artery ligation (Sandmann et al., 2001). This attenuation was also detected by pretreatment with a calpain inhibitor from 3 days before coronary artery ligation to 2 weeks after surgery by quantifying calpain and calpastatin levels after separation of these enzymes by high-performance liquid chromatography (Sandmann et al., 2002). Therefore, they suggested an important role for the activation of calpain in the myocardium after coronary artery ligation. Our findings are partly comparable to theirs.

In conclusion, degradation of α- and β-sarcoglycans and dystrophin in the right ventricle occurred in rats with failing heart following left ventricular myocardial infarction, which degradation was associated with enhanced cytosolic proteolysis in the right ventricle, probably mediated by calpain. Long-term treatment of the left coronary artery-ligated rat with ACE inhibitor or AT1 receptor blocker may restore α-sarcoglycan and preserve dystrophin, leading to preservation of cardiac cell membrane integrity and contractile function. The beneficial effects of ACE inhibitor and AT1 receptor blocker on preservation of the myocardial dystrophin glycoprotein complex may be attributed to attenuation of the left coronary artery ligation-induced increase in the cytosolic proteolysis mediated by activation of calpain. The findings of this present study are relevant to the ACE inhibitor and AT1 receptor blocker therapy for cardiac hypertrophy and chronic heart failure.

References

Badorff, C., Lee, G.-H., Lamphear, B.J., Martone, M.E., Campbell, K.P., Rhoads, R.E., Knowlton, K.U., 1999. Enteroviral protease 2A cleaves dystrophin: evidence of cytoskeletal disruption in an acquired cardiomyopathy. Nat. Med. 5, 320–326.

Capasso, J.M., Li, P., Meggs, L.G., Herman, M.V., Anversa, P., 1994. Efficacy of angiotensin-converting enzyme inhibition and AT1 receptor blockade on cardiac pump performance after myocardial infarction in rats. J. Cardiovasc. Pharmacol. 23, 584–593.

Daniels, M.C., Keller, R.S., de Tombe, P.P., 2001. Losartan prevents contractile dysfunction in rat myocardium after left ventricular myocardial infarction. Am. J. Physiol., Heart Circ. Physiol. 281, H2150–H2158.

Feng, J., Yan, J., Buzin, C.H., Towbin, J.A., Sommer, S.S., 2002. Mutations in the dystrophin gene are associated with sporadic dilated cardiomyopathy. Mol. Genet. Metab. 77, 119–126.

Hanada, H., Yoshida, T., Pan, Y., Iwata, Y., Nishimura, M., Shigekawa, M., 1997. mRNA expression and cDNA sequences of β- and γ-sarcoglycans are normal in cardiomyopathic hamster heart. Biol. Pharm. Bull. 20, 134–137.

Hoogerwaard, E.M., Bakker, E., Ippel, P.F., Oosterwijk, J.C., Majoor-Krakauer, D.F., Leschot, N.J., Van Essen, A.J., Brunner, H.G., van der Wouw, P.A., Wilde, A.A., de Visser, M., 1999. Signs and symptoms of Duchenne muscular dystrophy and Becker muscular dystrophy among carries in the Netherlands: a cohort study. Lancet 353, 2116–2119.

Kunkel, L.M., 1986. Analysis of deletions in DNA from patients with Becker and Duchenne muscular dystrophy. Nature 11, 73–77.

McKelvie, R.S., Yusuf, S., Pericak, D., Avezum, A., Burns, R.J., Probstfield, J., Tsuyuki, R.T., White, M., Rouleau, J., Latini, R., Maggioni, A., Young, J., Pougue, J., 1997. Comparison of candesartan, enalapril, and their combination in congestive heart failure: randomized evaluation of strategies for left ventricular dysfunction (RESOLVD) pilot study. The RESOLVD pilot study investigators. Circulation 100, 1056–1064.

McMahon, K.K., 1989. Developmental changes of the G proteins–muscarinic cholinergic receptor interactions in rat heart. J. Pharmacol. Exp. Ther. 251, 372–377.

Melacini, P., Fanin, M., Duggan, D.J., Freda, M.P., Berardinelli, A., Danieli, G.A., Barchitta, A., Hoffman, E.P., Dalla Volta, S., Angelini, C., 1999. Heart involvement in muscular dystrophies due to sarcoglycan gene mutations. Muscle Nerve 22, 473–479.

Noguchi, S., Wakabayashi, E., Imamura, M., Yoshida, M., Ozawa, E., 1999. Developmental expression of sarcoglycan gene products in cultured myocytes. Biochem. Biophys. Res. Commun. 262, 88–93.

Ozawa, E., Noguchi, S., Mizuno, Y., Hagiwara, Y., Yoshida, M., 1998. From dystrophinopathy to sarcoglycanopathy: evolution of a concept of muscular dystrophy. Muscle Nerve 21, 421–438.

Pfeffer, J.M., Pfeffer, M.A., Braunwald, E., 1987. Hemodynamic benefits and prolonged survival with long-term captopril therapy in rats with myocardial infarction and heart failure. Circulation 75, I149–I155.

Pfeffer, M.A., Braunwald, E., Moye, L.A., Basta, L., Brown Jr., E.J., Cuddy, T.E., Davis, B.R., Geltman, E.M., Goldman, S., Flaker, G.C., Klein, M., Lamas, G.A., Packer, M., Rouleau, J., Rouleau, J.L., Rutherford, J., Wertheimer, J.H., Hawkins, C.M., 1992. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. Results of the survival and ventricular enlargement trial. The SAVE investigators. N. Engl. J. Med. 327, 669–677.

Sanbe, A., Tanonaka, K., Hanaoka, Y., Katoh, T., Takeo, S., 1993. Regional energy metabolism of failing heart following myocardial infarction. J. Mol. Cell. Cardiol. 25, 995–1013.

Sanbe, A., Tanonaka, K., Kobayashi, R., Takeo, S., 1995. Effects of long-term therapy with ACE inhibitors, captopril, enalapril and trandolapril, on myocardial energy metabolism in rats with heart failure following myocardial infarction. J. Mol. Cell. Cardiol. 27, 2209–2222.

Sandmann, S., Yu, M., Unger, T., 2001. Transcriptional and translational regulation of calpain in the rat heart after myocardial infarction—effects of AT1 and AT2 receptor antagonists and ACE inhibitor. Br. J. Pharmacol. 132, 767–777.

Sandmann, S., Prenzel, F., Shaw, L., Schauer, R., Unger, T., 2002. Activity profile of calpains I and II in chronically infarcted rat myocardium—influence of the calpain inhibitor CAL 9961. Br. J. Pharmacol. 135, 1951–1958.

Sweet, C.S., Ludden, C.T., Stabilito, I.I., Emmert, S.E., Heyse, J.F., 1988. Beneficial effects of milrinone and enalapril on long-term survival of rats with healed myocardial infarction. Eur. J. Pharmacol. 147, 29–37.

Takahashi, M., Tanonaka, K., Yoshida, H., Oikawa, R., Koshimizu, M., Daicho, T., Toyo-oka, T., Takeo, S., 2005. Effects of ACE inhibitor and AT1 blocker on dystrophin-related proteins and calpain in failing heart. Cardiovasc. Res. 65, 356–365.

Tanonaka, K., Toga, W., Yoshida, H., Furuhama, K., Takeo, S., 2001. Effect of long-term treatment with trandolapril on Hsp72 and Hsp73 induction of the failing heart following myocardial infarction. Br. J. Pharmacol. 134, 969–976.

Vatta, M., Stetson, S.J., Perez-Verdia, A., Entman, M.L., Noon, G.P., Torre-Amione, G., Bowles, N.E., Towbin, J.A., 2002. Molecular remodeling of dystrophin in patients with end-stage cardiomyopathies and reversal in patients on assistance-device therapy. Lancet 359, 936–941.

Yoshida, H., Takahashi, M., Tanonaka, K., Maki, T., Nasa, Y., Takeo, S., 2001a. Effects of ACE inhibition and angiotensin II type 1 receptor blockade on cardiac function and G proteins in rats with chronic heart failure. Br. J. Pharmacol. 134, 150–160.

Yoshida, H., Tanonaka, K., Miyamoto, Y., Abe, T., Takahashi, M., Anand-Srivastava, M.B., Takeo, S., 2001b. Characterization of cardiac myocyte and tissue β-adrenergic signal transduction in rats with heart failure. Cardiovasc. Res. 50, 34–45.

Yoshida, H., Takahashi, M., Koshimizu, M., Tanonaka, K., Oikawa, R., Toyo-oka, T., Takeo, S., 2003. Decrease in sarcoglycans and dystrophin in failing heart following acute myocardial infarction. Cardiovasc. Res. 59, 419–427.