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Effects of long-term treatment with trandolapril on sarcoplasmic reticulum function of cardiac muscle in rats with chronic heart failure following myocardial infarction

Fuminari Yamaguchi, Atsushi Sanbe & [1]Satoshi Takeo

Department of Pharmacology, Tokyo University of Pharmacy and Life Science, Hachioji, Japan 192-03

1 Calcium transport activity of isolated cardiac sarcoplasmic reticulum (SR) including Ca^{2+} uptake and release is decreased in animals with chronic heart failure (CHF) following myocardial infarction. The present study was undertaken to determine whether an angiotensin converting enzyme (ACE) inhibitor, trandolapril, improves cardiac sarcoplasmic reticular function in animals with CHF following myocardial infarction.

2 CHF was induced by left coronary artery ligation in rats, which resulted in an infarction of approximately 45% of the left ventricle. Aortic flow and cardiac output index were decreased, and left ventricular end-diastolic pressure was increased 8 weeks after the operation, suggesting the development of CHF.

3 The developed force transients of cardiac skinned fibres of the rats with CHF were decreased when the skinned fibre was preloaded for 0.25–1 min with 10^{-5} M Ca^{2+} (48–88%) and when preloaded with 10^{-6} M Ca^{2+} and then exposed to 0.1–1 mM caffeine (45–93%).

4 The [^{3}H]-ryanodine-binding activity in SR-enriched fractions was reduced by 23% in the CHF group. These results suggest that the amount of Ca^{2+} released from SR is decreased due to a reduced rate of SR Ca^{2+} uptake and a downregulation of the SR Ca^{2+}-release channel.

5 Rats were treated orally with 3 mg kg^{-1} day^{-1} trandolapril from the 2nd to the 8th week after the coronary artery ligation. Treatment with trandolapril attenuated the reduction in aortic flow and cardiac output index and the increase in left ventricular end-diastolic pressure, and improved the developed force transients of the skinned fibre of the animal with CHF without causing a reduction of infarct size. Treatment with trandolapril also attenuated the reduction in ryanodine receptor density in the viable left ventricle of the rat with CHF.

6 It is concluded that long-term treatment with trandolapril attenuates cardiac SR dysfunction in rats with CHF and that the mechanism underlying this effect is, at least in part, attributed to prevention of downregulation of Ca^{2+} release channel.

Keywords: ACE inhibitor; heart failure; ryanodine receptor; sarcoplasmic reticulum; skinned fibre; trandolapril

Introduction

The cardiac sarcoplasmic reticulum (SR) plays a key role in the excitation-contraction coupling by regulating beat-to-beat intracellular Ca^{2+} transients of the heart. Therefore, a dysfunction of the SR may be a potential factor of contractile failure. Several investigators have demonstrated varying degrees of defects in SR Ca^{2+} transport activities in different types of heart diseases and failing hearts, such as a decrease in Ca^{2+} uptake activity in rats with heart failure following acute myocardial infarction (Afzal & Dhalla, 1992) and alterations in SR Ca^{2+} pumping activity in dogs with heart failure, due to rapid pacing or idiopathic dilated cardiomyopathy (Cory *et al*., 1993). Most of these studies have used isolated SR-enriched fractions and no investigation has dealt with the function of the intact SR and myocardium.

Long-term treatment with angiotensin converting enzyme (ACE) inhibitors has been demonstrated to improve cardiac function and mortality in man and animals and chronic heart failure (CHF) (Pfeffer *et al*., 1985; 1987; The SOLVD Investigators, 1991; 1992). Several mechanisms have been considered to play a role in this effect, including decreases in preload and afterload (Emmert *et al*., 1987; Raya *et al*., 1989), a restoration of reduced baroreflex sensitivity (Packer, 1988; Deck *et al*., 1992), attenuation in decreased cardiac β-adrenoceptor density (Sanbe & Takeo, 1995), and an inhibition of tissue and plasma renin-angiotensin system activation (Fabis *et al*., 1990; Hirsh *et al*., 1991; Yamagishi *et al*., 1993). An improvement of altered SR function is a possible mechanism for the benefit of ACE inhibitor therapy. However, there are few studies investigating the effects of ACE inhibitors on cardiac SR function in animals with CHF. The present study was undertaken to examine the effect of long-term treatment with an ACE inhibitor, trandolapril, on cardiac SR function in rats with CHF following myocardial infarction. This agent is a prodrug of trandolaprilat which has ACE inhibiting ability, and its potency has been shown to be 3–10 times higher than enarapril with respect to hypotensive effects and ACE inhibiting ability (Ohmura *et al*., 1985; Sakonjo *et al*., 1993).

Methods

Male Wistar rats weighing 220–240 g (SLC, Shizuoka, Japan) were used in the present study. The animals were fed standard rat chow and tap water *ad libitum*, maintained at 23 ± 1°C with a constant humidity of 55 ± 5%, and maintained with a cycle of 12 h of light and 12 h of dark according to the Guidelines of Experimental Animal Care issued by Prime Minister's Office of

[1]Author for correspondence at: Department of Pharmacology, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, 192-03, Japan.

Japan. The protocol of the study was approved by the Animal Care and Use Committee of the University.

Coronary artery ligation

Myocardial infarction was produced by occlusion of the left coronary artery according to the method described previously (Sanbe *et al*., 1993; Sanbe & Takeo, 1995). The animals were anaesthetized with pentobarbitone sodium (45 mg kg^{-1}, i.p.) and artificially ventilated with air. P_{O_2}, P_{CO_2} and pH of animal under surgery were 97.4 ± 5.7 mmHg, 40.0 ± 1.1 mmHg and 7.44 ± 0.02 ($n = 6$), respectively.

The skin was incised along with the left sternal border, and the fourth rib was cut proximal to the sternum. The pericardial sac was perforated and the heart was exteriorized through the intercostal space. The left coronary artery was ligated approximately 2 mm from its origin with a suture of 5-0 silk strong. The heart was repositioned in the chest and the wound was sutured with strings. Twenty-four hours after the operation, the rats were lightly anaesthetized with diethylether and their electrocardiograms (lead I) were monitored. The rats that showed a large Q-wave (> 1 mV) were considered to have developed acute myocardial infarction and used for the following experiments. Among 80 operated animals, 22 animals died within 24 h and 7 animals within 1 week after the operation. Forty-seven operated rats were used for the following studies. Five rats that had an infarct size of less than 35% in left ventricle were eliminated from this study. Forty-one sham-operated rats without the coronary artery ligation were treated similarly. Haemodynamic assessments, skinned fibre studies and receptor binding assays were performed 8 weeks after the operation. Eight, age-matched control animals were used to determine direct effects of trandolaprilat on cardiac SR function in the skinned fibre study.

Treatment with ACE inhibitor

Rats were treated orally with 3 mg kg^{-1} body weight of trandolapril once daily from 2nd to 8th week after the operation. In previous studies, it was demonstrated that long-term treatment with 3 mg kg^{-1} trandolapril improved cardiac function in rats with CHF (Sanbe & Takeo, 1995; Sanbe *et al*., 1995a). Trandolapril was suspended in 0.25% sodium carboxymethyl cellulose (CMC-Na). The drug solution in a volume of 1 ml kg^{-1} body weight was injected into the stomach through a probe.

The first series of experiments: measurements of haemodynamic parameters followed by determination of infarct area

Haemodynamic parameters of sham-operated and coronary artery-ligated (CAL) animals with or without trandolapril treatment were determined according to the method described previously (Sanbe *et al*., 1993). Eight weeks after the operation, the animals were anaesthetized with nitrous oxide, oxygen (3 : 1) and 2.5% halothane. Anaesthesia was continued with a gas mixture of nitrous oxide and oxygen (3 : 1) containing 0.5% halothane at the flow rate of 1.2 l min^{-1} through a mask loosely placed on the nose. In a preliminary study, we analysed the blood gas of the operating animal under the present experimental conditions. The P_{O_2}, P_{CO_2} and pH were 101.5 ± 4.9 mmHg, 40.1 ± 1.4 mmHg and 7.45 ± 0.02 ($n = 5$), respectively. A microtip pressure transducer (model SPC 320, Miller Instrument, Houston, Texas) was introduced into the left ventricle through the right carotid artery to measure left ventricular systolic pressure (LVSP) and end-diastolic pressure (LVEDP) and its dP/dt by means of a pressure transducer (model AB-621G, Nihonkohden, Tokyo) and a differentiator (model ED-601G, Nihonkohden, Tokyo), respectively. The arterial blood pressure was measured through a cannula placed into the right femoral artery by means of another pressure transducer (model DX-360, Nihonkohden, Tokyo). Heart rate measurements were triggered from changes in arterial blood pressure (model AT-601G, Nihonkohden, Tokyo). After equilibration for 10 min of the setting, the parameters were recorded on a thermal pen recorder (model RTA-1200, Nihonkohden, Tokyo).

Measurements of infarct size After measurements of haemodynamic parameters, 50 mM KCl solution was intravenously injected. The heart was isolated and sectioned into 7 slices (1 mm thick) from the base to the apex of the heart in a plane parallel to the atrioventricular groove. The slices were stained at 37°C for 5 min with 1% 2, 3, 5-triphenyltetrazolium chloride (TTC) in a physiological saline. The infarct areas were determined according to the planimetric method. The estimation was based on determination of the epi- and endo-circumference of the infarct myocardium as described previously (Fletcher *et al*., 1981).

The second series of experiments: measurement of aortic flow followed by determination of infarct size

Another set of experiments was designed to measure the aortic blood flow, and the cardiac output was determined by the method described previously (Sanbe & Takeo, 1995). Eight weeks after the operation, the animals were anaesthetized with nitrous oxide, oxygen (3: 1) and 2.5% halothane. Then the rat was intubated and artificially respirated with a gas mixture of nitrous oxide and oxygen (3: 1) containing 0.5% halothane at the flow rate of 2.90 – 3.5 ml $cycle^{-1}$ (60 cycle min^{-1}). After the right thoracotomy, an electromagnetic flow meter with a diameter of 2 to 2.5 mm (model MFV-3100, Nihonkohden, Tokyo) was placed around the thoracic aorta and the blood flow was measured. During the measurement of aortic flow, arterial blood pressure was monitored through a cannula inserted into the femoral artery and the heart rate was measured through the arterial blood pressure recording in a manner similar to that described above. Cardiac output index was calculated by dividing aortic flow by body weight. In a preliminary study, we performed blood gas analysis of the experimental animals. The P_{O_2}, P_{CO_2} and pH were 98.5 ± 3.3 mmHg, 37.5 ± 1.6 mmHg and 7.38 ± 0.03 ($n = 5$), respectively. After measurement of aortic flow, 50 mM KCl solution was intravenously injected. Measurement of infarct size was carried out as described above.

The third series of experiments: skinned fibre study

Preparation and solutions In the third set of experiments, a skinned fibre study was performed. Eight weeks after the operation, rats were anaesthetized with pentobarbitone sodium (45 mg kg^{-1}, i.p.). After thoracotomy, the heart was rapidly removed, placed in relaxing solution which consisted of 130 mM potassium propionate, 20 mM Tris-maleate, 2 mM ethylene glycol-bis (β-aminoethyl ether)-*N*,*N*, *N*,′*N*,′-tetraacetic acid (EGTA), 4 mM ATP, 2 mM creatine phosphate, pH 6.8, and was gassed with 100% O_2 at 4°C ($P_{O_2} > 600$ mmHg). Small bundles of muscle fibres (approximately 100 μm wide and 1 mm long) were dissected free from two papillary muscles of the left ventricle. Then the preparations were treated with β-

escin for 60 min in the relaxing solution at 4°C. β-Escin is a component of saponin and has a milder action on the cell membrane than saponin (Kobayashi *et al*., 1989). The concentration of β-escin was 100 μM for Ca^{2+} uptake and release studies. The conditions for treatment with β-escin were similar to those described by others (Kobayashi *et al*., 1989). Buffered Ca^{2+} solutions were made by adding $CaCl_2$ to the relaxing solution containing EGTA. The free Ca^{2+} concentration was calculated according to the methods of Fabiato & Fabiato (1979) and Fabiato (1981).

SR Ca^{2+} uptake and release SR Ca^{2+} uptake and release of the skinned fibres were determined by the method of Endo (1979) with a minor modification. The preparations were skinned with 100 μM β-escin for 60 min at 4°C. After treatment with β-escin, the preparations were mounted in a small tissue bath designed for measurement of isometric tension and rapid change of the superfusate. Tension development was recorded on a thermal pen recorder through a carrier amplifier (model AG-621G, Nihonkohden, Tokyo) connected to a force-displacement transducer (model TB-612T, Nihonkoden, Tokyo). Resting tension was adjusted to 5 mg. After an equilibration period, the fibres were immersed in a high calcium solution (3×10^{-5} M Ca^{2+}) for measurement of the maximum contraction. After washing with relaxing solution, the fibres were exposed sequentially to (1) Ca^{2+}-loading solution (10^{-6} M or 10^{-5} M Ca^{2+}) for 0.25–5 min, (2) low EGTA solution (Ca^{2+}-free relaxing solution containing 0.1 mM EGTA), and finally, (3) caffeine solution (low EGTA relaxing solution containing 0.1–25 mM caffeine). Since the concentration of EGTA in the caffeine solution is very low (weak buffer for Ca^{2+}), a small amount of Ca^{2+} released from the SR by caffeine can raise the Ca^{2+} concentration around the myofilaments and produce measurable force responses. Ca^{2+}, which causes contraction of the myofilaments, quickly diffuses out of the fibre into the bathing solution and then is chelated by free EGTA; thus the caffeine response is transient. The amount of Ca^{2+} released from the SR was estimated by caffeine-induced transient tension development. After a single treatment with 25 mM caffeine, the maximum contraction was observed, leaving the SR essentially depleted of Ca^{2+} (Tomita *et al*., 1994). Reapplication of caffeine produced no contraction. Thus, 25 mM caffeine was used to induce maximum Ca^{2+} release from the SR. Since we observed that the 25 mM caffeine-induced contraction reached steady state levels with Ca^{2+}-loading time of 4 min, 4 min Ca^{2+} loading was chosen for caffeine-induced contraction in the SR Ca^{2+}-release study. We repeated the Ca^{2+} loading-releasing procedure at an interval of 10 min. The magnitude of caffeine-induced contraction was stabilized after several (2–3 times) Ca^{2+} loading-releasing procedures. After a stabilization period, the experimental sequence was performed.

In vitro *effects of trandolaprilat on myocardial SR function* To examine the direct effects of trandolaprilat, an active form of trandolapril, on SR Ca^{2+} uptake and release of cardiac muscle, the skinned fibres were exposed to Ca^{2+}-loading solution for the Ca^{2+} uptake study and caffeine solution for the Ca^{2+} release study in the presence and absence of 100 μM trandolaprilat. The fibres obtained from control rats were used in this experiment, and the developed contractions were compared with those of the fibres without trandolaprilat treatment. The concentration of trandolaprilat used for this study was decided on the basis of the findings in a previous study from our laboratory that 30 to 100 μM of this agent were capable of exerting cardioprotective effects in ischaemic/reperfused rat hearts (Tanonaka *et al*., 1996).

The fourth series of experiments: ryanodine-binding study

Preparation of SR-enriched fraction In the fourth set of experiments, a ryanodine-binding study was carried out. Eight weeks after coronary artery ligation, the rats were anaesthetized with pentobarbitone sodium (45 mg kg^{-1}, i.p.). Hearts were quickly removed and rinsed in ice cold buffer A consisting of 200 mM sucrose, 200 mM Tris-HCl, 0.4 mM $CaCl_2$, pH 7.0. After removal of atria and connective tissue, the ventricles were separated to three sections, scar tissue, the remaining viable left ventricle including interventricular septum and the right ventricle. After being weighed, the remaining left ventricle was homogenized in 10 vol g^{-1} wet weight of buffer A with a Polytron homogenizer (PT-10, Kinematica, Lucerne, Switzerland) with two 15 s periods at the submaximal speed. The homogenates were filtered through four layers of gauze. SR-enriched microsomal fraction was prepared according to the method of Sapp & Howlett (1994) with some modifications. Briefly, the homogenate was centrifuged at $9{,}000 \times g$ for 30 min at 4°C, then the supernatant fluid was centrifuged at $100{,}000 \times g$ for 60 min. The final pellet was resuspended in 2.0 ml buffer A and used for binding assays. Protein concentrations of the samples were determined by the method of Lowry *et al*. (1951), with bovine serum albumin as standard.

Radioligand binding assay [^{3}H]-ryanodine-binding assay was conducted according to the method of Cory *et al*. (1993) with a minor modification. Approximately 100 μg of protein and different concentrations of [^{3}H]-ryanodine ranging from 0.05 to 5 nM were incubated at 37°C for 120 min in a total volume of 0.5 ml buffer B containing 1 M KCl, 50 mM HEPES and 0.01 mM $CaCl_2$, pH 7.4. The reaction was terminated by rapid filtration under reduced pressure through glass-fibre filters (GC50, ADVANTEC, Tokyo). Filters were washed three times with 2 ml of ice-cold buffer B, placed in vials, and their radioactivities were counted by liquid-scintillation spectroscopy (LSC-1000, Aloka, Tokyo). Specific binding was calculated by subtracting nonspecific binding activity, defined as that seen in the presence of [^{3}H]-ryanodine plus 500 nM unlabelled ryanodine, from total binding activity obtained in the presence of [^{3}H]-ryanodine alone. The numbers of animals used in the four series of experiments as above are shown in Table 1.

Materials

β-Escin was obtained from Sigma Chemical Company. [^{3}H]-ryanodine (62.8 Ci $mmol^{-1}$) was obtained from Du pont-New England Nuclear and unlabelled ryanodine was purchased from Wako Pure Chemical. Trandolapril and trandolaprilat were kindly given from Japan-Russel Co Ltd.

Statistical analysis

Data are expressed as means ± s.e.mean. Statistical significance was estimated by two-way ANOVA followed by Bonferroni/Dunn's *t* test or Student's *t* test. Differences with a probability of less than 5% were considered significant ($P < 0.05$).

Results

Haemodynamics and infarct size

Effects of long-term treatment with trandolapril on the haemodynamics and infarct size of CAL rats are shown in Table 2. The CAL rats showed decreases in body weight and $\pm$dP/dt and an increase in LVEDP after the operation. Treatment with trandolapril significantly attenuated the increase in LVEDP, but not the changes in body weight and $\pm$dP/dt.

In another set of experiments, aortic flow of sham-operated and CAL rats was measured and their cardiac output was determined (Figure 1). Mean arterial blood pressure and heart rate of the animals under the experimental conditions were similar to those observed in the first set of the haemodynamic studies described above. Aortic flow and cardiac output index of the CAL rats were significantly decreased 8 weeks after the operation. Treatment with trandolapril significantly attenuated the reduction in aortic flow and cardiac output index of the CAL rats. Haemodynamic parameters as above of the CAL animal treated with the vehicle containing 0.25% CMC-Na did not differ from those of the vehicle-untreated CAL animal (data not shown), suggesting that CMC-Na does not modify pathophysiological alterations of CAL animals.

Left ventricular infarct areas are shown in Table 2. As described in the Methods section, the rats with less than 35% in infarct area of left ventricle were excluded from the present study. The infarct areas of the CAL rat ranged from 40 to 49% of the total left ventricle in the present study. There was no infarction in the left ventricle of sham-operated rats and in the right ventricles of CAL and sham-operated rats. The infarct size was not altered by treatment with trandolapril.

Skinned fibres study

The ability of SR to take up and release Ca^{2+} in sham-operated and CAL rats was examined. At first, we measured the maximal tension development under high calcium-loading conditions (3×10^{-5} M free Ca^{2+}). Due to a high concentration of Ca^{2+} in the cytosol, participation of SR in this contraction is minimal. The contraction represents the maximal activation of the contractile proteins. Then we measured 25 mM caffeine-induced contraction under 10^{-6} M Ca^{2+}-loading for 4 min (SR participates in this contraction) and compared the ratios of the caffeine-induced contraction to the maximum contraction with high calcium (3×10^{-5} M free Ca^{2+}) solution in the three groups. The ratios were not significantly different between the

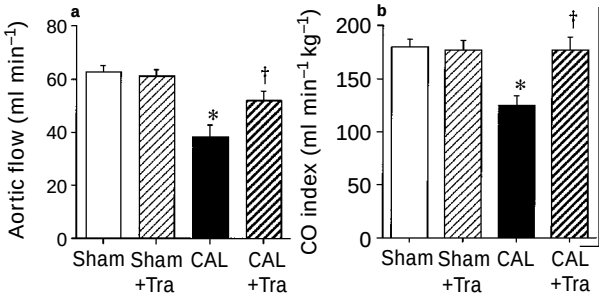

Figure 1 (a) Aortic flow and (b) cardiac output index (CO index) of the sham-operated rats (sham) and the coronary artery-ligated rats without (CAL) and with trandolapril treatment (sham + Tra and CAL + Tra). The infarct areas of the CAL rats in the absence and presence of trandolapril treatment were 44.7 $\pm$ 1.3% ($n = 6$) and 45.2 $\pm$ 1.2% ($n = 6$), respectively. Each column represents the mean $\pm$ s.e.mean of 5 to 6 experiments. * and † Significantly different from the corresponding sham-operated and coronary artery-ligated groups, respectively ($P < 0.05$).

Table 1 The numbers of animals used in the present study

	Control	*Sham*	*Sham + Tra*	*CAL*	*CAL + Tra*	*Total*
(1) Measurements of haemodynamics followed by determination of infarct size		4	6	7	6	23
(2) Measurement of aortic flow followed by determination of infarct size		5	5	6	6	22
(3) Skinned fibre study		7	5	7	6	25
in vitro effects of trandolaprilat	8					8
(4) Ryanodine binding assay		5	4	5	4	18
Total	8	21	20	25	22	96

Animals were subjected to coronary artery litigation and sham operation, and then untreated (CAL and sham) or treated with trandolapril (CAL + Tra and sham + Tra). To examine the direct effects of trandolaprilat, an active form of trandolapril, rats without any operation were used (control).

Table 2 Changes in cardiac and haemodynamic parameters and infarct size in rats with coronary artery-ligated (CAL) and sham-operated (sham) rats at the 8th week after the operation

	BW (g)	*HR* (beats min^{-1})	*MAP* (mmHg)	*LVSP* (mmHg)	$+dP/dt$ (mmHg s^{-1})	$-$dP/dt (mmHg s^{-1})	*LVEDP* (mmHg)	*Infarct size* (%)
Sham	350 $\pm$ 5	374 $\pm$ 6	104 $\pm$ 3	130 $\pm$ 5	6250 $\pm$ 450	7025 $\pm$ 248	2 $\pm$ 1	–
Sham + Tra	308 $\pm$ 5 *	384 $\pm$ 3	86 $\pm$ 2 *	101 $\pm$ 1 *	5283 $\pm$ 127	6050 $\pm$ 121	3 $\pm$ 1	
CAL	294 $\pm$ 10 *	387 $\pm$ 7	96 $\pm$ 4	107 $\pm$ 3 *	4357 $\pm$ 199 *	3314 $\pm$ 168 *	31 $\pm$ 1 *	44.1 $\pm$ 1.3
CAL + Tra	289 $\pm$ 14	362 $\pm$ 16	78 $\pm$ 5 †	92 $\pm$ 3 †	4200 $\pm$ 552 *	3467 $\pm$ 546 *	21 $\pm$ 2 *, †	46.0 $\pm$ 1.3

Each value represents the mean $\pm$ s.e.mean of 4 (sham), 7 (CAL) and 6 (sham + Tra, CAL + Tra) experiments. * and † Significantly different from the corresponding sham-operated (sham) and CAL (CAL) groups, respectively ($P < 0.05$). The abbreviations: BW, body weight; HR, heart rate; MAP, mean arterial pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure.

sham-operated and CAL rats 8 weeks after the operation; for example, the ratio of the sham-operated group was $68.8 \pm 3.0\%$, the CAL group was $74.1 \pm 3.5\%$, and the trandolapril-treated, CAL group was $71.1 \pm 4.0\%$ ($n = 7$, 7, 6, respectively) 8 weeks after the operations. This finding suggests that a difference in the developed tension among the three groups under these conditions is due to alterations of SR function.

SR Ca^{2+} uptake The ability of SR to take up Ca^{2+} at various Ca^{2+}-loading times was examined. The fibres were bathed in the 10^{-5} M Ca^{2+}-loading solution for various times ranging from 0.25–5 min, washed out with the low EGTA-relaxing solution, and then exposed to the low EGTA-25 mM caffeine solution (Figure 2). Data are expressed as percentages of the developed force transient to that induced by 25 mM caffeine solution 5 min after Ca^{2+}-loading. Figure 3 shows the time course of changes in caffeine-induced contraction at different Ca^{2+}-loading times with a solution containing 10^{-5} M Ca^{2+}, in the sham-operated and CAL rats. The caffeine-induced contraction was significantly reduced following Ca^{2+}-loading for 0.25, 0.5 and 1 min with 10^{-5} M Ca^{2+}-loading solution in the CAL group compared with the sham-operated group. Treatment with trandolapril significantly attenuated the reduced contraction following Ca^{2+}-loading for 0.25, 0.5 and 1 min with 10^{-5} M Ca^{2+}-loading solution in the CAL group. The caffeine-induced contractions of sham-operated animals treated with trandolapril were not different from those of the sham-operated group (data not shown).

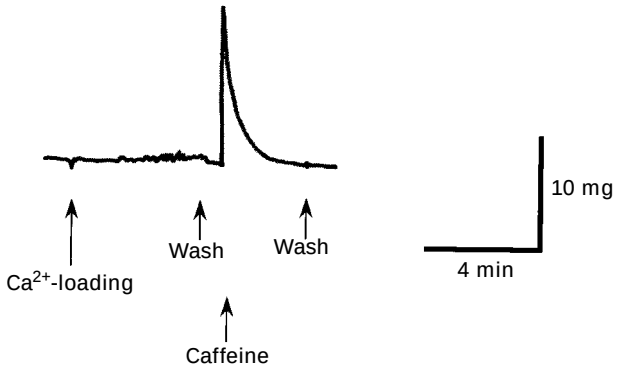

Figure 2 Typical trace of 25 mM caffeine-induced contraction following 10^{-6} M Ca^{2+} loading in the skinned fibres. The skinned fibre was loaded with 10^{-6} M Ca^{2+} at the arrow of Ca^{2+} loading, and then washed with Ca^{2+}-free solution at the arrow of 'wash'. Caffeine at a concentration of 25 mM was applied to the skinned fibre at the arrow of 'Caffeine'.

SR Ca^{2+} release To determine the ability of SR to release Ca^{2+}, we examined contractions induced by various concentrations of caffeine ranging from 0.1 to 25 mM. The fibres were bathed in 10^{-6} M Ca^{2+}-loading solution for 4 min, washed with the low EGTA-relaxing solution, and then exposed to solutions containing low EGTA and different concentrations of caffeine. The developed force transient is expressed as a percentage of that induced by 25 mM caffeine solution following 4 min of Ca^{2+}-loading. The developed force transient was reduced by lowering concentrations of caffeine solution in both sham-operated and CAL groups (Figure 4). The extent of the reduction was greater in the CAL group in the presence of 0.1, 0.5, 1 and 5 mM caffeine solutions. Treatment with trandolapril significantly attenuated the reduced contraction of the CAL group in the presence of 0.1, 0.5 and 1 mM caffeine solutions. The contractions of sham-operated animals treated with trandolapril were not different from those of the sham-operated group (data not shown).

In vitro *effects of trandolaprilat* Direct effects of 100 μM trandolaprilat on the contractions of skinned fibres were shown in Figure 5. There were no differences in the contractions of skinned fibres between treated and untreated groups in SR Ca^{2+} uptake and release studies.

Ryanodine binding assay

Figure 6 shows [^{3}H]-ryanodine-binding activities of the SR-enriched fraction of the sham-operated and CAL rat hearts with and without trandolapril treatment. Saturation binding data were fitted to non-linear curve-fitting techniques allowing the inference of values for affinity (K_d) and total receptor density (B_{max}) of [^{3}H]-ryanodine binding. These data

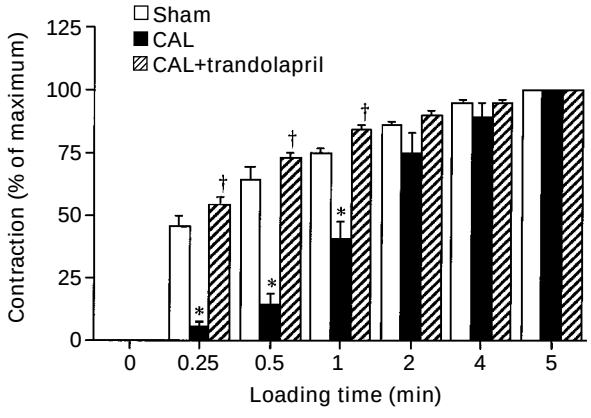

Figure 3 The 25 mM caffeine-induced contraction of skinned fibres of the myocardium of the sham-operated rat (sham) and the coronary artery-ligated rats (CAL) in the absence and presence of trandolapril treatment at various Ca^{2+} loading times measured following 10^{-5} M Ca^{2+}-loading. Each value represents the mean ± s.e.mean of 5 to 7 experiments. * and † Significantly different from sham-operated and coronary artery-ligated groups, respectively ($P < 0.05$).

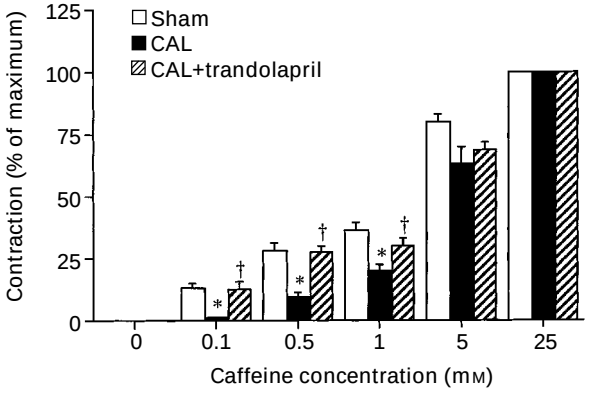

Figure 4 The caffeine-induced contraction of skinned fibres of the myocardium of the sham-operated rat (sham) and the coronary artery-ligated rats (CAL) in the absence and presence of trandolapril treatment at various concentrations of caffeine, measured following 4 min of Ca^{2+}-loading. Each value represents the mean ± s.e.mean of 5 to 7 experiments. * and † Significantly different from sham-operated and coronary artery-ligated groups, respectively ($P < 0.05$).

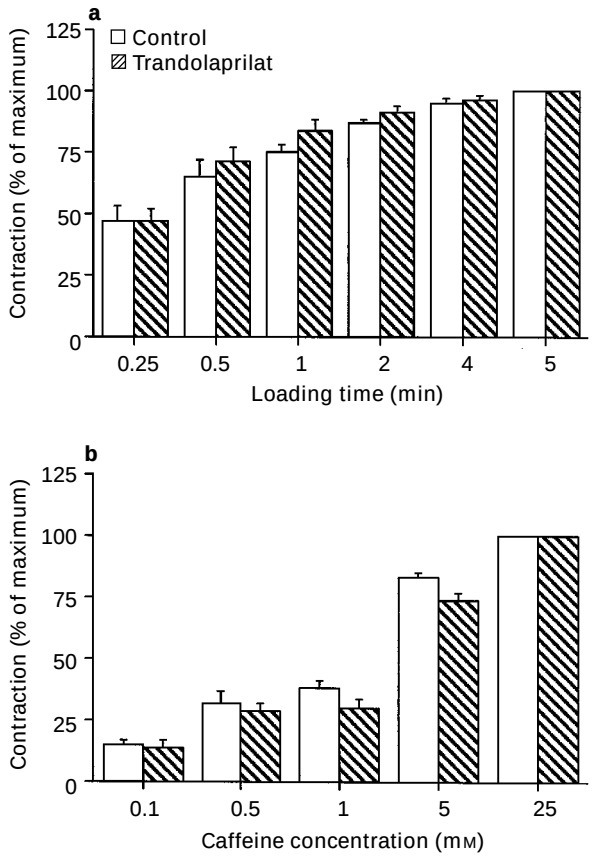

Figure 5 Direct effects of trandolaprilat on sarcoplasmic reticulum (SR) Ca^{2+} uptake (a) and Ca^{2+} release (b) activities of skinned fibres. The developed contractions were measured in the presence or absence (control) of trandolaprilat in skinned fibres of age-matched control rats. Each value represents the mean ± s.e.mean of 4 experiments.

Table 3 Ryanodine receptor density in the microsomal fraction of the left ventricle of sham-operated rats (sham) and the remaining left ventricle of coronary artery-ligated rats (CAL) at the 8th week after the operation

Groups	K_d *(nM)*	B_{max} *(fmol mg^{-1}* protein)
Sham	0.83 ± 0.05	685.43 ± 28.19
Sham + Tra	0.80 ± 0.09	651.38 ± 27.53
CAL	0.73 ± 0.07	525.76 ± 8.90 *
CAL + Tra	0.77 ± 0.06	681.47 ± 31.07 †

Each value represents the mean ± s.e.mean of 5 (sham and CAL) and 4 (sham + Tra and CAL + Tra) experiments. * and † Significantly different from the corresponding sham-operated (sham) and CAL (CAL) groups, respectively ($P<0.05$). The abbreviations: B_{max}; maximal binding capacity, K_d; dissociation constant.

were also transformed to Scatchard plots for visual comparison. As shown in Table 3, the K_d of the SR-enriched microsomal fraction did not differ between the sham-operated rat and CAL rats with and without trandolapril treatment. In contrast, the B_{max} of the CAL group, as shown in Table 3, was decreased (22% in SR-enriched fraction, $P<0.05$). Long-term treatment with trandolapril prevented the decrease in the maximum density of binding site for [^{3}H]-ryanodine of the SR-enriched fraction of the remaining left ventricle of the CAL rats. The protein yields of the SR-enriched microsomal fraction

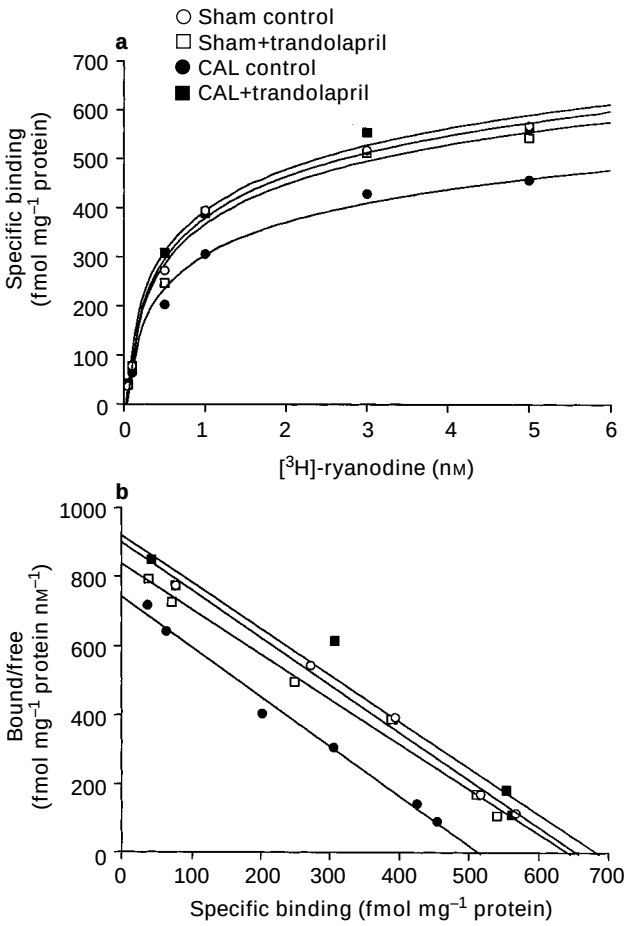

Figure 6 (a) Specific binding activity of [^{3}H]-ryanodine to the left ventricular sarcoplasmic reticulum (SR)-enriched fractions from sham-operated rats treated or untreated (sham control) with trandolapril, and the remaining left ventricular SR-enriched fractions from coronary artery-ligated rats treated or untreated (CAL control) with trandolapril. (b) Scatchard plot.

used for ryanodine binding studies were 0.15 ± 0.01 and 0.14 ± 0.01% for the sham-operated rat hearts in the absence ($n=5$) and presence of trandolapril treatment ($n=4$), respectively, and 0.12 ± 0.01 and 0.14 ± 0.01% for the CAL rat hearts in the absence ($n=5$) and presence of trandolapril treatment ($n=4$), respectively. There were no significant differences in the protein yields between the four groups.

Discussion

In the present study we showed that most of the CAL rats had approximately 45% infarction of the left ventricle 8 weeks after the operation. The CAL animals also revealed a decreased LVSP, elevated LVEDP and reduced aortic flow and cardiac output index. The CAL animals showed evidence of ascites, liver and lung congestion. In a previous study, we observed increases in lung oedema and ascites in the same model, in addition to alterations in the haemodynamic parameters described above (Sanbe *et al.*, 1993). These observations suggest that chronic heart failure with low cardiac output had developed by this period. Long-term treatment with an ACE inhibitor, trandolapril, attenuated the increase in LVEDP and reduction in cardiac output index, typical symptoms of chronic heart failure. Thus, treatment is effective in improving cardiac dysfunction

of the animals with CHF. Several studies have shown a marked hypotensive effect of the ACE inhibitor at this dose: in the rat a single dose of trandolapril 3 mg kg^{-1} decreased blood pressure (BP) from 180 mmHg to 130 mmHg, whereas chronic treatment with 3 mg kg^{-1} decrease BP from 130 mmHg to 100 mmHg (Ohmura *et al*., 1985). The magnitude of the hypotensive effects of this agent is in good agreement with that in the present study, suggesting that the hypotensive effects contribute to the improvement of haemodynamics in rats with CHF.

A decrease in the developed force transient of the skinned fibre of the CAL rat was observed when 10^{-5} M Ca^{2+} was loaded for 0.25 to 1 min, but not for 2 min or more. This suggests that the rate of Ca^{2+} uptake by the SR was decreased in animals with CHF. Afzal & Dhalla (1992) have shown that the Ca^{2+} uptake activity of cardiac SR vesicles isolated from the CAL rat was decreased when determined in the presence of 10 to 100 μM of extravesicular free Ca^{2+} and oxalate, an agent enhancing the precipitation of free Ca^{2+} into SR. Our findings are comparable with the latter results, although differences in the preparations and conditions used for study should be taken into consideration. Treatment with trandolapril completely reversed the decreased rate of SR Ca^{2+} uptake in the skinned fibre. Since a decrease in SR Ca^{2+} uptake is considered to play an important role in Ca^{2+} handling in the calcium-induced calcium release mechanism (Endo, 1979), our findings suggest that chronic treatment with trandolapril is capable of improving the impaired Ca^{2+} handling ability of cardiac SR in CHF.

The ability of cardiac SR to release Ca^{2+} was also examined in the present study. For this purpose, the sensitivity of cardiac SR of the CAL animal to concentrations of caffeine ranging from 0 to 25 mM was examined. Since 25 mM caffeine can release all SR Ca^{2+}, contraction in the presence of 25 mM caffeine was taken as the maximal Ca^{2+} release from SR, whereas contraction evoked in the presence of lower concentrations of caffeine may represent a partial release of Ca^{2+} from SR. The results in the present study showed that the developed force transient induced by lower concentrations (0.1 to 1 mM) of caffeine was reduced in the CAL rats. This suggests that the ability of SR to release Ca^{2+} is reduced in the animals with CHF. Since Ca^{2+} release from SR is the major mechanism by which cytoplasmic Ca^{2+} concentration is elevated during cardiac contraction (Feher & Fabiato, 1990), the decrease in contraction of the skinned fibre of the CAL rat is at least in part attributed to a defect in the ability to release Ca^{2+} from SR. Treatment with trandolapril appreciably reversed this effect, which may contribute to the recovery of cardiac contractile dysfunction in the CAL animals.

To characterize the ability to release Ca^{2+} further, the Ca^{2+} release channel of the left ventricle of the sham-operated and CAL rats was analysed. It is believed that ryanodine specifically acts on SR Ca^{2+} release channels (ryanodine receptor) in the calcium-induced release mechanism and irreversibly inhibits them in an open state, since ryanodine completely inhibits caffeine-induced muscle contraction without inhibiting IP_3-induced muscle contraction (Meissner, 1986; Rousseau *et al*., 1987). In the present study, we observed that total ryanodine receptor density (B_{max}), but not receptor affinity (K_d), was reduced in SR-enriched microsomal fraction of the CAL rat 8 weeks after the operation. This indicates that the ryanodine receptors were downregulated whereas a qualitative alteration of the receptor did not occur at this time. Several investigators have described changes in cardiac SR Ca^{2+} release channel density in various experimental animals, including prehypertrophic cardiomyopathic hamsters (Sapp & Howlett, 1994), hypertrophied rats induced by chronic pressure (Ohkusa *et al*., 1994) and volume overload (Hisamatsu *et al*., 1994), and dogs with rapid pacing-induced heart failure (Cory *et al*., 1993). Thus, our results accord with the hypothesis that the downregulation of ryanodine receptors is common in animals with cardiac contractile dysfunction. Treatment with trandolapril completely reversed the B_{max} of SR-enriched microsomal fraction, indicating that the downregulation of the Ca^{2+} release channel of cardiac SR was restored by this treatment. Such an effect may be attributed to a restoration of the ability to release Ca^{2+} from SR, and eventually it will lead to recovery of cardiac contractile failure in animals with CHF.

Our findings in the present study definitely showed beneficial effects of trandolapril on SR dysfunction in animals with CHF, including the recovery of the abilities for Ca^{2+} uptake and Ca^{2+} release and the restoration of B_{max} of ryanodine binding receptors. However, the mechanisms underlying the effects cannot be elucidated from the results in the present study. Several studies have shown the benefit of ACE inhibitor treatment on the myocardium in animals with cardiac failure or hypertrophy, such as normalization in the expression of gene coding for the SR Ca^{2+}-ATPase (Anger *et al* 1995; Zierhut *et al*., 1996), prevention of the development of cardiac hypertrophy, attenuation of the delayed repolarization of cardiac action potential (Thollon *et al*., 1989) and blockade of DNA synthesis in the remaining left ventricle (Van Krimpen *et al*., 1991). The former two findings might be related to the pathophysiological alterations of the SR function in the present study. However, the latter two findings appear to be unrelated directly to the recovery of SR function in the present study. Furthermore, we demonstrated in a previous study that long-term treatment with trandolapril reversed the downregulation of β-adrenoceptors (Sanbe & Takeo, 1995) and improved myocardial energy metabolism (Sanbe *et al*., 1995a) in the same animal model of CHF. Thus, a possible mechanism for the effects of trandolapril on animals with CHF may be attributed not to a specific action but to a comprehensive effect of the agent. Several studies have shown effects of ACE inhibitor treatment on humoral factors, including attenuation of an increase in plasma noradrenaline concentration (Cody *et al*., 1982; Kleber & Doering, 1991), a rise in cardiac ACE activity (Sanbe *et al*., 1995b) and increase in plasma concentration of ANP and BNP in man (Kawahara *et al*., 1989; Crozier *et al*., 1989; Yoshimura *et al*., 1994). Prevention of changes in humoral factors by long-term treatment with ACE inhibitors might play an important role in the recovery of cardiac SR function and thus lead to an improvement of cardiac contractile force in animals with CHF. Although the mechanism underlying the benefit of long-term treatment with trandolapril remains unclear, the present study provides evidence for an additional site, cardiac SR, of action for long-term treatment with an ACE inhibitor.

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