

Beneficial effects of sampatrilat, a novel vasopeptidase inhibitor, on cardiac remodeling and function of rats with chronic heart failure following left coronary artery ligation

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A list of nonstandard abbreviations : ACE, angiotensin-converting enzyme; ANP, atrial natriuretic peptide; CAL, coronary artery ligation; CHF, chronic heart failure; DME, Dulbecco's modified Eagle's medium ; FCS, fetal calf serum; NEP, neutral endopeptidase; LVEDP, left ventricular end-diastolic pressure; Sham, sham-operated; HR, heart rate; MAP, mean arterial pressure; LVSP, left ventricular systolic pressure; +LVdp/dt, +LV dp/dt max; -LV dp/dt, -LV dp/dt max, RAA: renin-angiotensin-aldosterone, and RVSP, right ventricular systolic pressure:

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ABSTRACT

Sampatrilat is a novel vasopeptidase inhibitor that may offer a greater benefit than traditional ACE inhibitors in the treatment of chronic heart failure (CHF). The present study was undertaken to determine whether sampatrilat improves hemodynamic function and cardiac remodeling through a direct action on the failing heart in rats with CHF following left coronary artery ligation (CAL). Sampatrilat (30 mg/kg/day) was administered orally to the animals from the 1st to 6th week after the operation. Sampatrilat reduced the mortality of the rats with CAL (20% vs. 57% for untreated rats). Treatment with sampatrilat for 5 weeks suppressed tissue ACE and neutral endopeptidase (NEP) activities. Sampatrilat did not affect the arterial blood pressure whereas it attenuated the CAL-induced increases in the left ventricular end-diastolic pressure, heart weight, and collagen content of the viable left ventricle. To assess the direct effects of sampatrilat on collagen synthesis, we measured the incorporation of [^3H]-proline into cultured cardiac fibroblasts. Sampatrilat at concentrations that inhibited NEP activity *in vitro* augmented the atrial natriuretic peptide (ANP)-induced decrease in [^3H]-proline incorporation by the cells. In addition, sampatrilat prevented the angiotensin I-induced increase in [^3H]-proline incorporation, whereas captopril did not. The results suggest that long-term treatment with sampatrilat regresses cardiac remodeling in rats with CAL, which is associated with improvement of hemodynamic function. The mechanism by which sampatrilat improved cardiac remodeling may be attributable to the direct inhibition of cardiac fibrosis, possibly acting through cardiac natriuretic peptide system.

Key words: sampatrilat, chronic heart failure, cardiac remodeling, collagen, vasopeptidase inhibitor, neutral endopeptidase

Cardiac remodeling is often accompanied by an abnormal increase in collagen deposition that severely impairs myocardial contractility in patients and animals with chronic heart failure (CHF) (Dixon *et al.*, 1996; Pelouch *et al.*, 1993; Sabbah *et al.*, 1995; Schaper and Speiser, 1992). Thus, cardiac remodeling appears to be a critical pathophysiological event in CHF, and the inhibition of excessive cardiac fibrosis can be expected to have a favorable effect on contractile function in failing hearts. Several neurohumoral factors derived from the sympathetic nervous, renin-angiotensin-aldosterone (RAA), and endothelin systems are activated in the failing heart as well as in the systemic circulation (Sakai *et al.*, 1996; Yamagishi *et al.*, 1993). Since these humoral factors are responsible for the induction of myocardial remodeling, inactivation of these factors would exert salutary effects on cardiac remodeling. Indeed, the administration of ACE inhibitors or AT1 blockers was proved to be successful strategies for achieving regression of myocardial remodeling in CHF.

Neutral endopeptidase (NEP), an enzyme responsible for the degradation of natriuretic peptides, and ACE are both zinc metalloproteases. Vasoepitidase inhibitor, a new type of pharmaceutical drug, has a unique property of inhibiting both NEP and ACE activities and thereby leads to activation of the natriuretic peptide system and suppression of the RAA system (Bralet and Schwartz, 2001; Corti *et al.*, 2001). Omapatrilat, a novel vasoepitidase inhibitor, has been shown to be an effective antihypertensive agent and to have a great potential for the treatment of congestive heart failure (Nathisuwan and Talbert, 2002; Nawarskas *et al.*, 2001). Vasoepitidase inhibitor is expected to be therapeutically beneficial in the regulation of humoral factors in patients and animals with CHF and to be more effective in this respect than ACE inhibitors alone (Lapointe and Rouleau, 2002). In fact, the IMPRESS (Inhibition of Metalloproteinase in a Randomized Exercise and Symptoms Study in Heart Failure) trial showed that omapatrilat improved clinical status and lowered the incidence of the combined end point of mortality or admission for worsening heart failure more effectively than lisinopril, an ACE inhibitor (Rouleau *et al.*, 2000). However, more recent reports showed no significant difference between omapatrilat and traditional ACE inhibitors such as enalapril and captopril with respect to the therapeutic benefit in patients with CHF (Packer *et al.* OVERTURE trial, 2002) and in animal models with CHF (Lapointe *et al.*, 2002). Therefore, the potential role of NEP inhibition in addition to ACE inhibition is not yet fully understood.

Sampatrilat (UK81252) is also a vasoepitidase inhibitor. Its K_i values (8.0 nM for NEP and 1.2 nM for ACE) are almost similar to those of omapatrilat (K_i for NEP and ACE, 8.9 nM and 0.5 nM, respectively; Bralet and Schwartz, 1991). It was reported that sampatrilat produced sustained

antihypertensive action in hypertensive subjects who were resistant to the antihypertensive action of ACE inhibitor monotherapy (Norton *et al.*, 1999). As far as we know, there are, however, no reports concerning the effects of sampatrilat on myocardial remodeling and hemodynamic function in CHF.

The present study was designed to determine whether sampatrilat might affect cardiac remodeling and whether these effects might be associated with an improvement in survival and cardiac function. We used rats with CHF following coronary artery ligation (CAL) because this model has been shown to induce massive fibrosis in the myocardium, to cause deterioration of cardiac function, and to ultimately lead to death probably due to worsening of the symptoms of heart failure. To assess the direct effects of sampatrilat on cardiac fibrosis, we also determined the incorporation of [³H]-proline into cultured cardiac fibroblasts. The effects of sampatrilat were compared with those of thiorphan, a typical and relatively selective NEP inhibitor, and captopril, a typical ACE inhibitor, to characterize the pharmacological properties of this vasopeptidase inhibitor on cardiac tissues.

Experimental Procedures

Animals. Male Wistar rats weighing 220-240g (SLC, Hamamatsu, Japan) were used. All experiments were performed according to the Guidelines for Experimental Animal Care published by the National Institutes of Health. The experimental protocols were approved by the Animal Use and Care Committee of the Tokyo University of Pharmacy and Life Science.

Heart failure following left coronary artery ligation. Myocardial infarction was produced by CAL as described previously (Sanbe *et al.*, 1993). Briefly, the animals were anesthetized with pentobarbital sodium (45 mg/kg, i.p.), intubated, and artificially ventilated with air. The left coronary artery was ligated approximately 2 mm from its origin. A sham operation was also performed without CAL. Electrocardiograms were recorded 24 hours after the operation, and the animals that showed a large Q wave (>0.3 mV) were used in subsequent experiments.

Treatment with sampatrilat. For evaluation of the effects of long-term treatment with sampatrilat, both rats with CAL and sham-operated (Sham) rats were orally treated with 30 mg/kg of sampatrilat once daily for 5 weeks from the 1st to the 6th week after the operation. The dose used in the present

study was based on the data of our preliminary study; that is, the pressor responses to a bolus injection of angiotensin I (1-1,000 ng/kg) in anesthetized rats were significantly attenuated by pretreatment with sampatrilat at 10 and 30 mg/kg, p.o., 24 hrs before the measurement. The latter dose had a more profound effect than the former one without influencing the basal blood pressure.

Measurements of hemodynamic parameters and myocardial infarct size. Six weeks after the operation, heart rate (HR), mean arterial pressure (MAP), left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), +LV dp/dt max (+LVdp/dt), -LV dp/dt max (-LV dp/dt), and right ventricular systolic pressure (RVSP) of Sham and CAL animals with or without sampatrilat treatment were measured according to the methods described previously (Maki *et al.*, 2001). The animals were anesthetized with nitrous oxide/oxygen (3:1) and 0.5-2.5% halothane. LV and RV functions such as LVSP, LVEDP, LV \pm dp/dt, and RVSP were measured via a microtip pressure transducer (Miller Instrument). In a preliminary study, we analyzed the blood gas of the animal during the operation under the present experimental conditions. The PO₂, PCO₂, and pH were 102 \pm 5 mmHg, 40 \pm 2 mmHg and 7.45 \pm 0.02 (n=5), respectively. After measurements of hemodynamic 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 in a plane parallel to the atrioventricular groove. The 4th slice from the apex was stained with 1% 2,3,5-triphenyltetrazolium chloride in physiological saline to assess the myocardial infarct size. The infarct areas were determined according to the planimetric method (Maki *et al.*, 2001). Other slices were divided into 4 portions (scar, viable left ventricle, septum, and right ventricle) to determine tissue collagen content and NEP activity.

Measurement of tissue NEP and ACE activities. To assess the inhibitory potency of sampatrilat on the target enzymes, we measured the NEP activity in the kidney, lung, and heart, as well as the ACE activity in the lung of CAL and Sham rats. For the measurement of NEP activity, we employed a fluorimetric method using succinyl-Ala-Ala-Phe-aminomethylcoumarin (Sigma) as a synthetic substrate for NEP (Maki *et al.*, 2001). For the measurement of ACE activity, Hip-His-Leu (Sigma) was used as a synthetic substrate for ACE based on a modification of the methods described by others (Cheung and Cushman, 1973; Tsai and Peach, 1977).

Measurement of collagen content. Tissue collagen content was measured according to the method described previously (Yoshida *et al.*, 2001) with a collagen staining kit (Cosmo Bio Co.).

Cell culture. Rat cardiac fibroblasts were prepared as described previously (Maki *et al.*, 2000) with minor modification. Ventricles were separated and minced, and the pieces were dispersed in the balanced salt solution containing 0.1% collagenase type II and then digested. These steps were repeated 8 times. The isolated cells were resuspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS), and the suspension was plated onto culture dishes and incubated for 2 hrs. The dishes were then rinsed with the cultured medium, and the tightly adhered cells were allowed to reach confluence and then trypsinized and passaged at a dilution of 1:3. Cardiac fibroblasts at the second passage were plated at a density of 2.5×10^4 cells/well onto 24-well plates and allowed to grow to confluence before being used in the *in vitro* study.

Measurements of collagen and DNA synthesis and NEP activity in cultured cardiac fibroblasts.

The effects of sampatrilat on angiotensin I- and atrial natriuretic peptide (ANP)-induced changes in collagen synthesis in the cardiac fibroblasts were examined by assessing the incorporation of [3 H]-proline into the cells according to the method described previously (Maki *et al.*, 2000). After incubation in DMEM containing FCS, cardiac fibroblasts were maintained in serum-free DMEM for 24 hrs. The culture medium was replaced with fresh serum-free DMEM. Either angiotensin I or ANP was added to the cells in the presence and absence of sampatrilat, thiorphan, or captopril. To evaluate collagen and DNA synthesis, we incubated the cells for 24 hrs with [3 H]-proline (0.5 μ Ci/mL) and [3 H]-thymidine (0.5 μ Ci/mL), respectively. The radioactivities of the incorporated [3 H]-proline and [3 H]-thymidine were determined by using a liquid scintillation counter. NEP activity in cultured cardiac fibroblasts was determined after incubation for 24 hrs in the presence and absence of sampatrilat (0.1~10 μ M) according to the method described above.

Data Analysis. Analysis of survival after CAL was carried out by using the χ^2 -test. Other data were expressed as means \pm S.E.M. Statistical significance in the *in vivo* experiment was estimated by 2-way factorial ANOVA followed by Fisher's PLSD method. In *in vitro* studies, the statistical significance of differences between the drug-untreated groups (ANP or angiotensin I alone) and the treated groups was tested by 1-way ANOVA followed by the Dunnett method. *P* values of less than 0.05 were considered statistically significant.

Materials Sampatrilat was kindly provided by Pfizer Pharmaceutical Inc. Tokyo, Japan. Other

drugs used in this study and their sources were as follow: angiotensin I, angiotensin II, thiorphan, captopril, and ANP (Sigma Chemical Co., St. Louis, MO) and collagenase type II (Worthington Biochemical Corporation, Lakewood, NJ). Stock solutions of these drugs were held frozen until required. All solutions were freshly prepared daily before use and protected from light.

Results

Survival and myocardial infarction after CAL. Fifty-seven percent (8/14) of the untreated CAL rats died between the 1st and 6th week after CAL, whereas only 20% (2/10) of the sampatrilat-treated CAL rats died during this period ($P < 0.05$). This increase in survival was not due to differences in the size of the myocardial infarct, because the infarct size of the rats with CAL was not affected by the drug treatment (44 ± 3 vs. $45 \pm 3\%$ for the untreated CAL group).

Effects on tissue weight and hemodynamic parameters. Changes in body, heart, and lung weights (wt) and in hemodynamic parameters of rats with CAL and Sham rats are shown in Table 1. Lung wt/body wt and heart wt/body wt were increased by CAL, and the treatment with sampatrilat attenuated these changes. Mean arterial pressure and heart rate in rats with CAL and in Sham rats were not affected by treatment with sampatrilat. The increase in LVEDP of the rats with CAL was attenuated by treatment with sampatrilat (Fig. 1A). The increase in RVSP tended to be reversed by the treatment with sampatrilat (Fig. 1B).

Effects on cardiac collagen content. Changes in collagen content are shown in Fig. 2. A marked increase in collagen content was seen in the scar tissue of the rat with CAL as compared with that of the remaining LV of the rat with CAL and LV of Sham rats. An increase in collagen content was also observed in the viable LV and septum, but not in the RV, of the rat with CAL. These increases were reversed to Sham levels by the treatment with sampatrilat.

Effects on tissue NEP and ACE activities. Tissue NEP and ACE activities of rats with CAL and Sham rats were measured following chronic treatment with sampatrilat (Table 2). NEP activities in the scar, LV, septum, and RV of rats with CAL were lower than those in lung and kidney, and they were markedly inhibited by treatment with sampatrilat. The pulmonary ACE activities in sampatrilat-treated rats with CAL and Sham rats were significantly lower than those in the untreated rats.

Effects on NEP activity as well as collagen and DNA synthesis in cultured cardiac fibroblasts.

NEP activity was detected in the cultured cardiac fibroblasts (Table 3). *In vitro* treatment with sampatrilat inhibited the NEP activity in a concentration-dependent manner. Sampatrilat did not affect the basal [^3H]-proline uptake in the absence of humoral factors at any concentration used. Because the incorporation of [^3H]-proline into cardiac fibroblasts was attenuated by ANP (1~100 nM) and increased by 0.1 μM angiotensin I (data not shown), the effects of sampatrilat on [^3H]-proline and [^3H]-thymidine uptake were measured in the presence of either ANP (10 nM) or angiotensin I (100 nM). The ANP-induced decrease in [^3H]-proline uptake was augmented by treatment with sampatrilat (0.1~10 μM) concentration-dependently (Fig. 3A). Thiorphan also augmented the ANP-induced decrease in [^3H]-proline uptake (Fig. 3B), but captopril did not (Fig. 3C). Sampatrilat at the same concentrations as those that enhanced the ANP-induced decrease in collagen synthesis also attenuated the angiotensin I-induced increase in [^3H]-proline uptake (Fig. 4A). As expected, captopril (0.1~10 μM) attenuated the angiotensin I-induced increase in [^3H]-proline uptake (Fig. 4B), whereas thiorphan (0.1~10 μM) had no effect (Fig. 4C). The incorporation of [^3H]-thymidine into cardiac fibroblasts was decreased by treatment with ANP (10 nM), and sampatrilat enhanced this decrease (Fig. 5A). Sampatrilat concentration-dependently attenuated the angiotensin I-induced increase in [^3H]-thymidine uptake (Fig. 5B).

Discussion

In the *in vivo* experiments, we found that long-term treatment with sampatrilat prevented the increases in heart weight and cardiac collagen content of the rats with CAL, suggesting that sampatrilat attenuates the development of cardiac remodeling. This was associated with the increase in the survival rate and improvement of hemodynamic function of the rats with CAL. These are the first observations showing the beneficial effects of sampatrilat on the pathophysiological changes in CHF.

The increase in survival was achieved by a 5-week treatment with sampatrilat. Because sampatrilat did not reduce the systemic blood pressure, such survival benefit is not due to the depressive effects of sampatrilat on arterial blood pressure (ex. afterload pressure). In a similar model of CHF, long-term treatment with fasidotril, another vasopeptidase inhibitor, improved cardiac hypertrophy and survival without a reduction in blood pressure (Marie et al., 1999). It

must be noted that, in rats with CHF, long-term treatment with ACE inhibitors at the doses that reduced blood pressure increased the survival rate only in the rats with moderate infarction (20~35%) and not in those with severe infarction (>35%; Pfeffer *et al.*, 1985; Sweet *et al.*, 1987; Wollert *et al.*, 1994). In this study, we demonstrated that sampatrilat increased the survival rate even in rats with a large infarct (~45%). In cardiomyopathic hamsters, treatment with the vasopeptidase inhibitor omapatrilat for 32 weeks resulted in a greater increase in the survival rate (88%) as compared with that with captopril (48%; Trippodo *et al.*, 1999). Our findings suggest that sampatrilat is superior to ACE inhibitors with respect to survival in CHF, although we did not directly compare the effects of sampatrilat with those of an ACE inhibitor in *in vivo* experiments. More recent observation has revealed, however, that treatment with either captopril or omapatrilat had similar effects on cardiac function and survival in rats with CHF (Lapointe *et al.*, 2002). Since the K_i value of sampatrilat for NEP is 7-fold higher than that for ACE, it is unlikely that the activity of sampatrilat to inhibit NEP, as compared with that to inhibit ACE, preferentially contributes to its systemic actions. Nevertheless, the potential role of NEP inhibition should be taken into account in consideration of the mechanism of the beneficial effect of sampatrilat on CHF.

NEP inhibitors have been shown to reduce the metabolic clearance of ANP and to enhance its natriuretic and vasodilatory actions (Chen *et al.*, 1999). Our recent study demonstrated that a single administration of a NEP inhibitor, ONO-9902, suppressed the plasma and renal NEP activities and enhanced an increase in the plasma ANP concentration in rats with CAL (Maki *et al.*, 2001). Since long-term treatment with sampatrilat, like ONO-9902, suppressed the tissue NEP activity, this drug may also activate the natriuretic peptide system and thereby may enhance the diuretic and vasodilatory actions. Sampatrilat attenuated the CAL-induced increase in LVEDP, a sensitive index of the increase in preload pressure in failing hearts. The increase in lung and body weight ratio was reduced by treatment with sampatrilat, as was the case with ONO-9902 (Maki *et al.*, 2001). Although the lung weight of the treated rats was not reversed to the normal level, chronic NEP inhibition appears to have a beneficial effect on pulmonary congestion caused by CHF. Burrell *et al.* (2000) reported that S21402, a vasopeptidase inhibitor, may offer several advantages, such as enhanced sodium and water excretion and relief of pulmonary congestion in rats with CHF. Considering these observations, we suggest that sampatrilat prevented worsening of the symptoms of CHF, at least in part, due to chronic inhibition of NEP activity.

It is generally accepted that an excessive accumulation of extracellular matrix proteins such as collagen in the noninfarcted myocardium is a critical sign of ventricular remodeling following

myocardial infarction (Weber, 1997). In the present study, we showed that the amount of collagen in the viable LV was increased in the rat with CAL and that sampatrilat reduced the LV collagen deposition to the sham level. The expression of type I collagen mRNA is reported to be increased in the noninfarcted rat myocardium, and the sites of ACE binding anatomically coincide with those of this expression and fibrous tissue formation (Sun *et al.*, 1994; Weber *et al.*, 1997). In addition, the mRNA expression of type I collagen is activated by angiotensin II and inhibited by treatment with an ACE inhibitor or AT₁-blocker (Kim *et al.*, 1995; Linz *et al.*, 1995). Since the cardiac RAA system plays a critical role in the development of myocardial remodeling, we suggest that the activity to inhibit ACE largely contributes to the effect of sampatrilat on cardiac remodeling. It should be emphasized that long-term treatment with NEP inhibitor alone attenuated cardiac hypertrophy and fibrosis in SHR (Monopoli *et al.*, 1992; Pu and Schiffrin, 2001). NEP protein is present in hearts (Piedimonte *et al.*, 1994). Since NEP activity was detected in the myocardium of the rats with CAL and was markedly inhibited by long-term treatment with sampatrilat, inhibition of NEP activity may also contribute to the favorable effect of sampatrilat on cardiac remodeling. It is possible that sampatrilat directly acts on both ACE and NEP in the failing heart of rats with CAL and thereby ameliorates cardiac remodeling.

We observed no significant changes in the total heart weight between the rats with CAL and sham rats. Evaluation of total heart weight in the CAL rats is quite complicated in this model. Both weight and area of the LV free wall became smaller due to formation of the scar tissue, which caused a thinning of the infarct areas. In contrast, those of the septum and RV free wall became larger due to the compensatory hypertrophy. Since the total heart weight was estimated by the sum of the weights of the decreased LV free wall and the increased hypertrophied septum and viable left ventricle, it appeared to be unchanged. In addition, the cardiac collagen content increased in the rats with CAL. Consequently, the development of cardiac hypertrophy was apparent without a significant increase in total heart weight.

It remains unclear whether the regression in cardiac remodeling achieved by sampatrilat is secondary to the hemodynamic improvement in the systemic circulation or not. Thus, we examined NEP activity and, collagen and DNA synthesis in the presence or absence of sampatrilat in cultured cardiac fibroblasts. NEP activity detected in the cells was directly inhibited by *in vitro* treatment with sampatrilat. At the same concentration, sampatrilat augmented the suppressive effects of ANP on the synthesis of collagen and DNA. In addition, sampatrilat attenuated the angiotensin I-induced increase in collagen and DNA synthesis in cardiac fibroblasts. On the other hand, captopril did not affect the ANP-induced decrease in collagen

synthesis and thiorphan did not affect the angiotensin I-induced increase in collagen synthesis, either. These results suggested that local natriuretic peptide and RAA systems were present in the cardiac fibroblasts and that sampatrilat directly acted on both systems and thereby inhibited the cardiac remodeling independent of hemodynamic changes. The enhancement of the cardiac natriuretic peptide system, inhibition of the cardiac RAA system, and their synergistic effects afforded by sampatrilat may largely contribute to the regression of cardiac fibrosis in the rat with CAL.

A cause-effect relation between cardiac remodeling and survival remains to be established. Currently, it has been reported that the regression of cardiac remodeling caused by chronic inhibition of the RAA system is associated with an improvement in both survival rate and hemodynamics in animal models (Harada *et al.*, 1999; Schieffer *et al.*, 1994) and patients (Pfeffer *et al.*, 1997) with CHF. More recently, long-term treatment with an inhibitor of prolyl 4-hydroxylase, an essential enzyme in collagen synthesis, was reported to prevent interstitial fibrosis and to improve cardiac function without affecting afterload in rats with CHF (Nwogu *et al.*, 2001). These results suggest that the regression of cardiac remodeling may be achieved without affecting systemic hemodynamic function. It was also recently reported that omapatrilat improved both cardiac and vascular fibrosis in SHRSP (Pu and Schiffrin, 2001). Therefore, direct inhibition of interstitial collagen deposition in the failing hearts may be a plausible mechanism for the salutary effect of sampatrilat on cardiac remodeling in the rats with CHF.

In conclusion, we demonstrated that long-term treatment with sampatrilat improved cardiac function and cardiac remodeling of rats with CAL without affecting systemic blood pressure. The mechanism by which sampatrilat improved cardiac remodeling may be attributable to the direct inhibition of cardiac fibrosis.

Figure legends

Figure 1. Effects of long-term treatment with sampatrilat on changes in left ventricular end-diastolic pressure (LVEDP, A), and right ventricular systolic pressure (RVSP, B) of rats with coronary artery ligation (CAL) and sham-operated rats (Sham). Values represent the means \pm S.E.M. of 6 experiments. * $P < 0.05$ vs. sampatrilat-untreated Sham; # $P < 0.05$ vs. sampatrilat-untreated CAL.

Figure 2. Effects of long-term treatment with sampatrilat on changes in tissue collagen content of scar tissue (A), viable left ventricle (viable LV, B), septum (C), and right ventricle (RV, D) of rats with coronary artery ligation (CAL) and sham-operated rats (Sham). Values represent the means \pm S.E.M. of 6 experiments. * $P < 0.05$ vs. sampatrilat-untreated Sham; # $P < 0.05$ vs. sampatrilat-untreated CAL.

Figure 3. Effects of sampatrilat (A), thiorphan (B), and captopril (C) on ANP-induced changes in collagen synthesis in cultured cardiac fibroblasts. Values represent the means \pm S.E.M. of 6 experiments. * $P < 0.05$ vs. ANP alone.

Figure 4. Effects of sampatrilat (A), captopril (B), and thiorphan (C) on angiotensin I (Ang I)-induced changes in collagen synthesis in cultured cardiac fibroblasts. Values represent the means \pm S.E.M. of 6 experiments. * $P < 0.05$ vs. angiotensin I alone.

Figure 5. Effects of sampatrilat on ANP-induced (A) and angiotensin I (Ang I)-induced (B) changes in DNA synthesis in cultured cardiac fibroblasts. Values represent the means \pm S.E.M. of 6 experiments. * $P < 0.05$ vs. ANP or angiotensin I alone.

Table 1. Effects of long-term treatment with sampatrilat on changes in lung and heart weights, heart rate (HR), mean arterial pressure (MAP), left ventricular systolic pressure (LVSP), +LV dp/dt, and -LV dp/dt, of rats with left coronary artery ligation (CAL) and of sham-operated rats (Sham) at the 6th week after the operation

	Sham		CAL		P (ANOVA)		
	untreated	treated	untreated	treated	CAL	treated	inter
n	6	6	6	6			
Body wt (g)	301±4	282±7	254±12	258±11	0.0019	NS	NS
Lung wt (mg)	974±60	935±27	2493±193	1953±183 *	<0.001	0.0472	NS
Lung wt (mg)/body wt (g)	2.83±0.13	3.32±0.05	9.74±0.72	8.01±0.47 *	<0.0001	NS	0.0186
Heart wt (mg)	761±64	640±33	780±40	643±20 *	NS	0.0074	NS
Heart wt (mg)/body wt (g)	2.25±0.25	2.27±0.10	3.02±0.13	2.36±0.08 *	0.0143	NS	0.044
HR (beats/min)	397±6	395±3	399±3	402±9	NS	NS	NS
MAP (mmHg)	106±5	103±6	108±3	96±3	NS	NS	NS
LVSP (mmHg)	150±10	139±6	134±6	122±8	0.0477	NS	NS
+LV dp/dt (mmHg/sec)	11300±579	10633±303	6616±248	6533±961	<0.0001	NS	NS
-LV dp/dt (mmHg/sec)	8800±292	11266±597	4700±399	5000±785	<0.0001	0.0209	NS

Values represent the means±S.E.M. * P<0.05 vs. untreated CAL. Abbreviations : body wt, body weight; lung wt, lung weight; heart wt, heart weight. NS, not significant.

Table 2. Effects of long-term treatment with sampatrilat on changes in tissue neutral endopeptidase (NEP) and angiotensin-converting enzyme (ACE) activities of rats with left coronary artery ligation (CAL) and of sham-operated rats (Sham) at the 6th week after the operation

	Sham		CAL		P (ANOVA)		
	untreated	treated	untreated	treated	CAL	treated	inter
n	6	6	6	6			
EP activity (pmol/mg protein/min)							
scar			2.07±0.57	0.10±0.04*			
left ventricle	3.38±0.64	0.745±0.63*	1.61±0.32	<0.1 *	0.0029	<0.0001	NS
septum	0.94±0.36	0.83±0.21*	1.12±0.20	<0.1 *	NS	0.0146	0.0417
right ventricle	0.34±0.15	0.37±0.17	1.29±0.37	<0.1 *	NS	0.0001	0.0073
lung	83.3±9.5	44.9±6.7	49.1±3.0	29.0±2.0*	0.0005	0.0001	NS
kidney	3154±357	1293±184*	2196±289	288±99 *	0.0009	0.0021	NS
CE activity (nmol/mg protein/min)							
Lung	13.8±0.3	5.7±1.3*	4.2±0.5	1.9±0.4*	<0.0001	<0.0001	0.0014

Values represent the means±S.E.M. * P<0.05 vs. corresponding untreated group. NS means not significant.

Table 3. Effects of sampatrilat on NEP activity, collagen synthesis, and DNA synthesis in cultured cardiac fibroblasts.

	control	sampatrilat		
		0.1 μ M	1 μ M	10 μ M
NEP activity (pmol/mg protein/min)	18.98 \pm 1.84	18.10 \pm 1.54	7.06 \pm 0.83*	2.06 \pm 0.58*
[3 H]-proline uptake (% of control)	100.0 \pm 3.7	99.1 \pm 1.3	99.7 \pm 2.0	96.8 \pm 1.0
[3 H]-thymidine uptake (% of control)	100.0 \pm 2.5	100.8 \pm 3.2	99.9 \pm 3.4	102.0 \pm 3.6

Values represent means \pm S.E.M. of 8 measurements. * P <0.05 vs. control

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Fig. 1

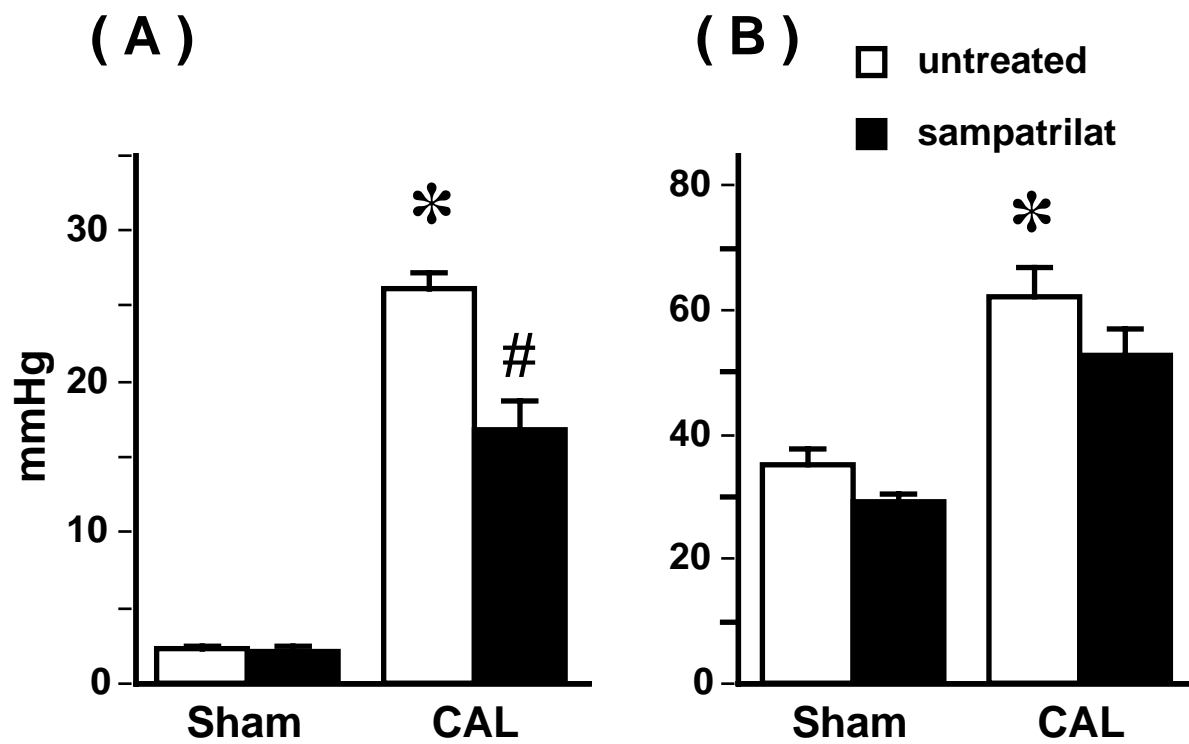


Fig. 2

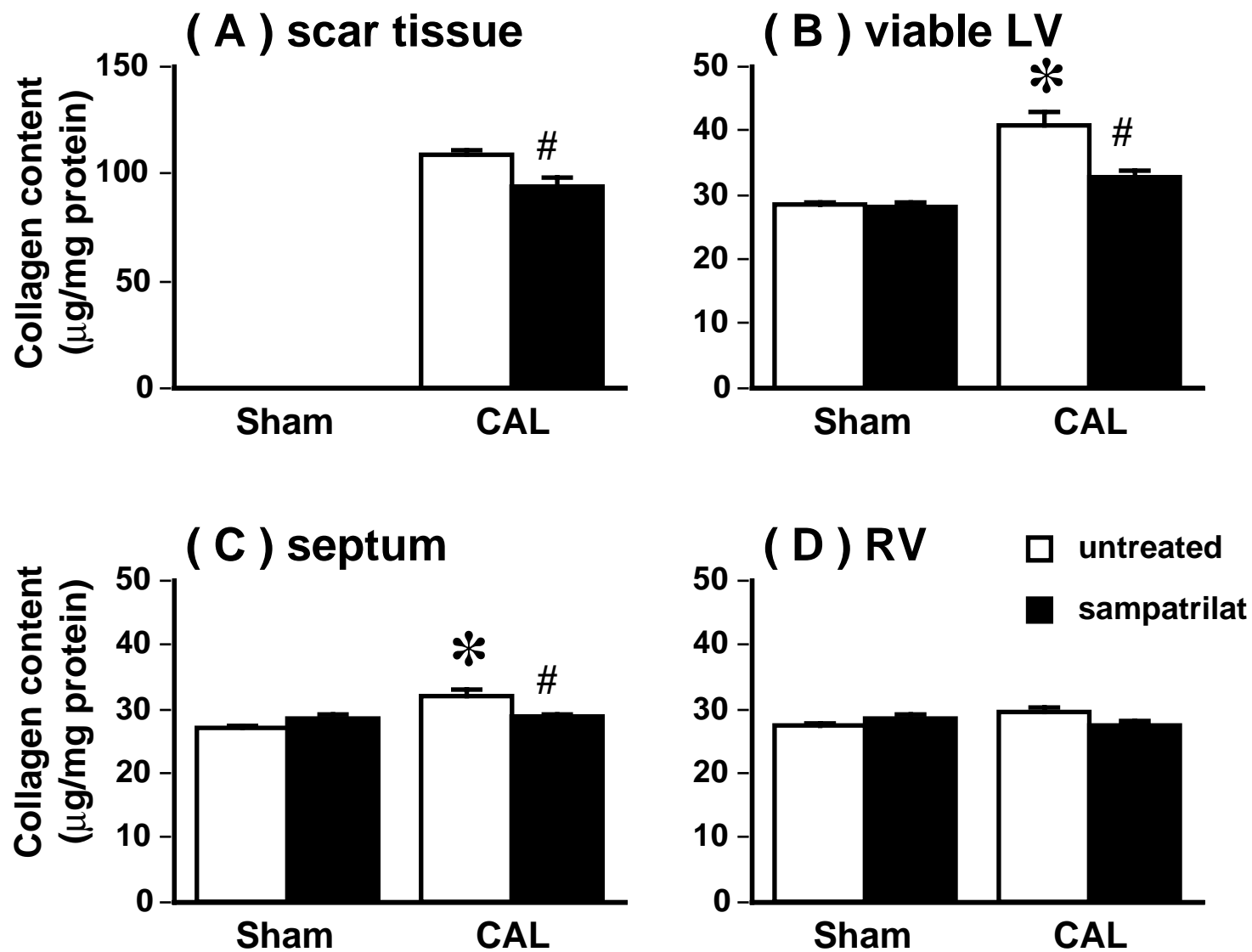


Fig. 3

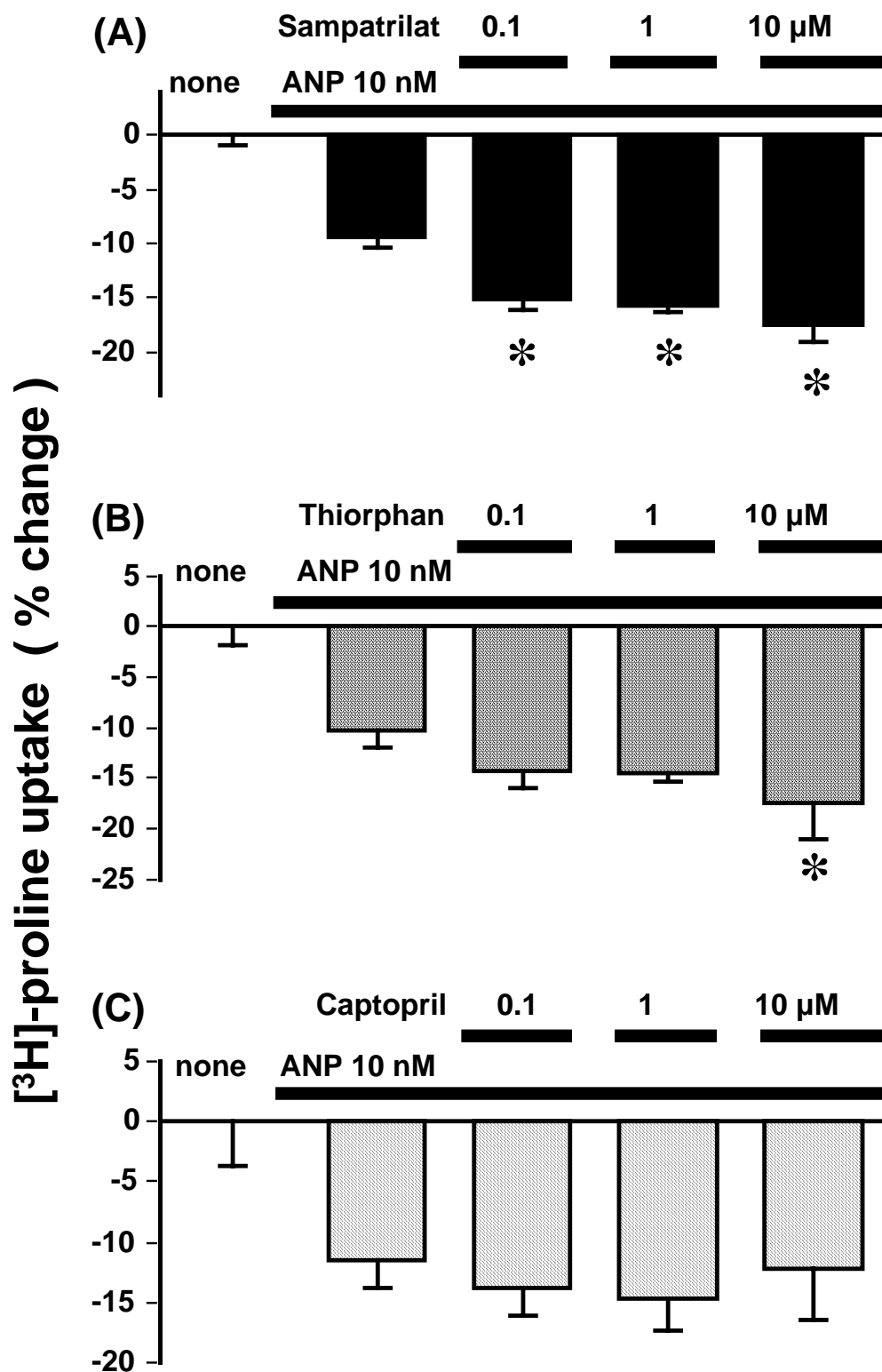


Fig. 4

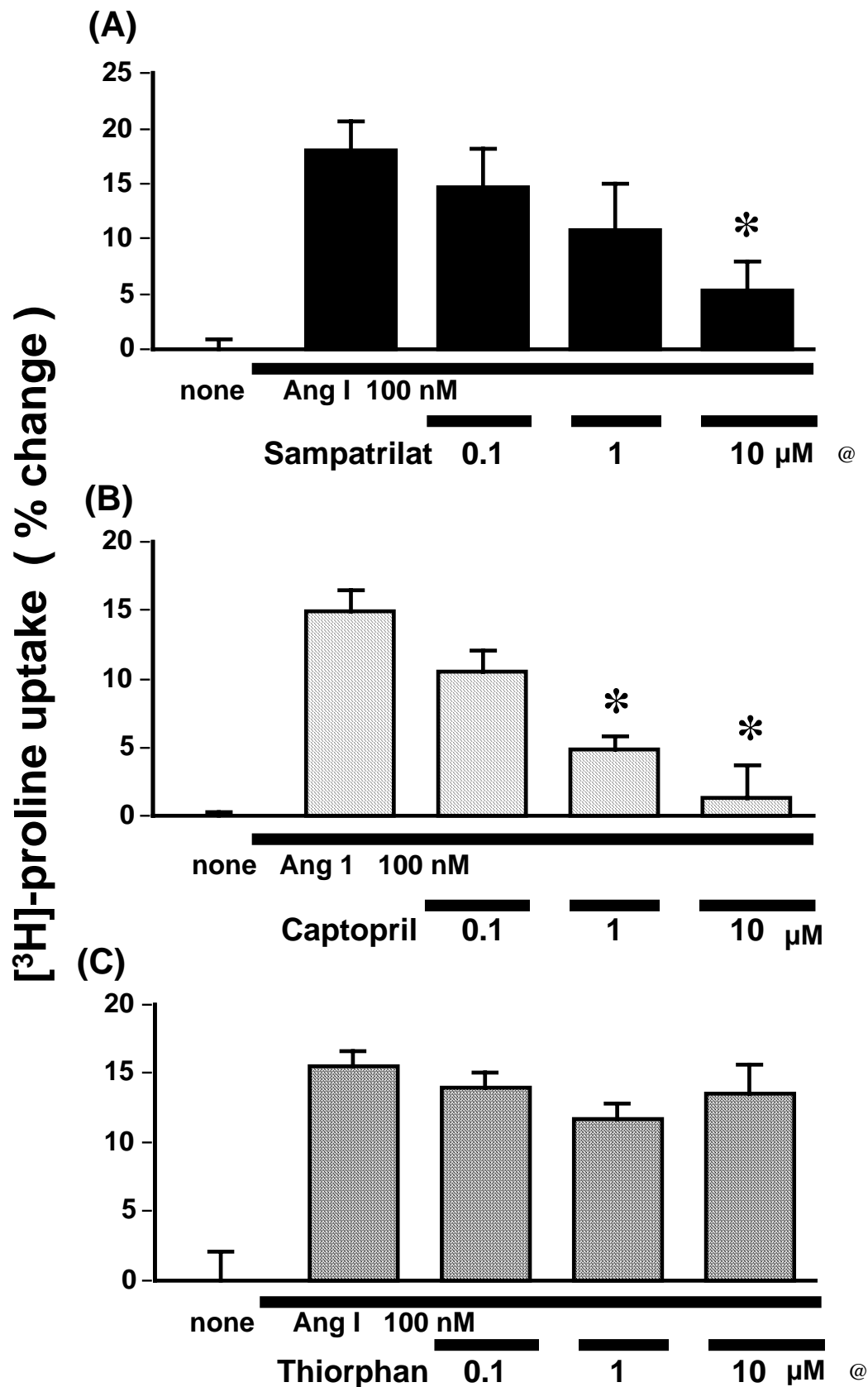


Fig. 5

