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Lack of correlation of hypotensive effects with prevention of cardiac hypertrophy by perindopril after ligation of rat coronary artery

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1 The present study was designed to test the hypothesis that beneficial effects of angiotensin converting enzyme (ACE)inhibitors are independent of a fall in blood pressure in rat experimental heart failure following coronary ligation.

2 The animals were assigned randomly to six groups; sham operation, controls subjected to coronary ligation (control), coronary ligation plus chronic treatment with ACE inhibitors at non- and hypotensive doses; perindopril (0.2 or 2 mg kg^{-1} day^{-1}) or enalapril (2 or 20 mg kg^{-1} day^{-1}) for three weeks starting one week after the ligation.

3 Systemic blood pressure was measured every week during the experiments. At the end of the treatments, cardiac function and heart weight (an index of myocardial hypertrophy) were determined. In the other animals, ACE activities in plasma and tissues including heart, kidney, lung and blood vessels were measured.

4 In the controls, cardiac ACE activity, weight of right ventricle and left ventricular end-diastolic pressure (LVEDP) were higher compared to those in the sham-operated animals four weeks after the coronary ligation. However, ACE activities were not changed in plasma, kidney, lung and aorta by ligation of the coronary artery.

5 The chronic treatment with perindopril at a dose of 0.2 mg kg^{-1} day^{-1} inhibited the increase in ACE activity in cardiac tissue and suppressed the right ventricular hypertrophy without affecting systemic haemodynamics. In contrast, enalapril at a dose of 20 mg kg^{-1} day^{-1}, but not 2 mg kg^{-1} day^{-1}, prevented the development of the right ventricular hypertrophy. Enalapril at 20 mg kg^{-1} day^{-1} also lowered systemic blood pressure.

6 There is no significant correlation between systemic blood pressure and right ventricular hypertrophy at the end of the treatment with perindopril ($r = 0.06$) or enalapril ($r = 0.1$).

7 These findings demonstrate that perindopril, an ACE inhibitor, prevents cardiac hypertrophy without affecting systemic blood pressure in the rat with heart failure after coronary ligation, and suggest that selective augmentation of ACE activity in cardiac tissue is involved in the progression of hypertrophy in this model.

Keywords: Angiotensin-converting enzyme; blood pressure; cardiac hypertrophy; coronary ligation; enalapril; perindopril

Introduction

Experimental myocardial infarction following ligation of the coronary artery results in compensatory hypertrophy in the non-infarcted myocardium. In the rat model of myocardial infarction, angiotensin converting enzyme (ACE) inhibitors, such as captopril and enalapril, prevent the progression of cardiac hypertrophy, though the exact mechanism of this prevention is unclear (Pfeffer *et al.*, 1985; 1988). The increase in pre- and afterload against the heart may favour the growth of myocytes. Indeed, ACE inhibitors are potent vasodilator agents and are likely to attenuate the pre- and afterload due to direct actions on haemodynamics. However, the reduction in blood pressure does not necessarily correlate with the prevention of cardiac hypertrophy since some classes of antihypertensive agents, such as calcium antagonists, do not significantly suppress the development of hypertrophy (Linz *et al.*, 1988; Kromer & Riegger, 1988). In the rat model of pressure overloaded cardiac hypertrophy by aortic stenosis, ACE inhibitors attenuate cardiac hypertrophy without affecting blood pressure (Linz *et al.*, 1989; Linz & Schölkens, 1992). Alternatively, earlier studies demonstrate that the renin-angiotensin system in the hearts may participate in the regulation of cardiac function and growth adaptation of the remaining viable myocytes (Campbel *et al.*, 1986; Hirsch *et al.*, 1991; Yamada *et al.*, 1991; Reiss *et al.*, 1993; Meggs *et al.*, 1993). Angiotensin II stimulates the growth of myocardium (Aceto *et al.*, 1990; Baker *et al.*, 1990) and also enhances the release of noradrenaline, which is another possible mediator of the cardiac hypertrophy via activation of α_1-adrenoceptors (Simpson, 1983). Furthermore, ACE inhibitors reduce the degradation of bradykinin in the heart (Baumgarten *et al.*, 1993; Noda *et al.*, 1993). This peptide may inhibit the cell growth due to release of nitric oxide by activation of kinin B_2 receptors (Farhy *et al.*, 1992; Linz & Schölkens, 1992). Taken in conjunction, these observations suggest that ACE inhibitors, independently of a fall in blood pressure, can prevent the progression of cardiac hypertrophy following coronary ligation. In the present study, the effects of chronic treatment with perindopril, a novel long-acting ACE inhibitor (Macfayden *et al.*, 1990), and enalapril at non-hypotensive doses were studied on cardiac hypertrophy after ligation of the coronary artery in rats.

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Methods

Animals

Animals in the present study were treated according to the guidelines for animal experimentation prepared by the Japanese Association for Laboratory Animals Science. Sprague-Dawley male rats (Nihon SLC, Shizuoka, Japan), weighing 250–300 g, were assigned to sham-operation or myocardial infarction group after an observation period of three weeks.

Myocardial infarction was produced by ligation of the left coronary artery as described by Selye *et al.* (1960) with a modification. Briefly, the animals were anaesthetized by inhalation of 2% halothane, followed by 0.5% halothane during artificial respiration. A left thoracotomy was performed at the fourth intercostal space. The heart was gently exteriorized to ligate the left coronary artery 2 mm from its origin. Then, the heart was replaced in its normal position and the chest was compressed to remove air from the pleural cavity and stitched with a single suture. Successful occlusion of the artery was confirmed by appearance of Q waves in the electrocardiogram (Leads I, II and aVL), indicating the presence of myocardial ischaemia (Pfeffer *et al.*, 1987; Sweet *et al.*, 1987; 1988; Raya *et al.*, 1989). In the sham-operation group, the animals underwent left thoracotomy without ligation of the coronary artery.

One week after the surgery, the animals were anaesthetized with 2% halothane to record electrocardiograms. The animals with Q waves in leads I, II and aVL were randomly assigned to one of five groups: (a) control treatment, and chronic treatment with (b) perindopril (0.2 mg kg^{-1} day^{-1}), (c) perindopril (2 mg kg^{-1} day^{-1}), (d) enalapril (2 mg kg^{-1} day^{-1}), and (e) enalapril (20 mg kg^{-1} day^{-1}). The animals were allowed free access to tap water (sham-operation and control treatment groups) or drinking water including perindopril or enalapril for three weeks. The concentration of perindopril was adjusted to be equivalent to a daily dose of 0.2 and 2 mg kg^{-1} and enalapril, 2 and 20 mg kg^{-1} day^{-1}. The rats were weighed every week to readjust the concentrations of the compounds in the drinking water. The drug solutions were made freshly every two days. Systolic arterial pressure and heart rate were measured weekly during the treatment by means of a tail-cuff probe connected to a pressure monitor (BP-98, Softron, Japan). At the end of the treatments, cardiac function, cardiac weight and myocardial infarction size were measured. In the other animals, plasma and tissue ACE activities were determined.

Cardiac function

At the end of the treatment, cardiac function was measured under anaesthesia with thiopentone. A micro-tip catheter transducer (Model SPR-407, 2F, Miller Instruments Inc., Houston, U.S.A.) was introduced into the left ventricle through the right carotid artery for the measurement of systolic and end-diastolic pressure and maximum positive dP/dt.

Ventricular weight and myocardial infarction size

After the study of cardiac function, hearts were rapidly removed. The hearts were separated into right and left ventricle including septum, and the wet weight of the ventricles was measured. The hearts from coronary-ligated rats were fixed in 10% buffered formalin solution for determination of myocardial infarction size. The left ventricles were trimmed and sliced transversely, parallel to the atrioventricular groove, in five sections of 1.5–2.0 mm from apex to base. The sliced sections were dehydrated in methyl alcohol, cleared with xylene, and embedded in paraffin. Then, sections were cut, 5 μm in thickness and stained with Elastica-VanGieson trichrome. These serial sections were mounted on slides for photography. Infarct size was measured by planimetry and calculated as the mean percentage ratio of infarction to total left ventricular circumference (Fletcher *et al.*, 1981).

Measurement of ACE activity

Under anaesthesia with thiopentone, blood was collected from the carotid artery to obtain plasma. Tissues (heart, lung, kidney and aorta) were removed after exsanguination, and homogenized in 0.3% Triton X solution. After centrifugation of the homogenates at 29,200 *g* for 15 min, the supernatant was used for measurement of ACE activity which was determined by a modified fluorometric method according to Unger *et al.* (1982), using hippuryl-L-histidine-L-leucine as a substrate. Briefly, the tissue homogenate and plasma sample (50 μl) were incubated in 400 μl of PBS (pH 8.0): 300 mM NaCl for 2 min at 37°C. Then, 50 μl of 16.7 mM substrate solution was added to the reaction mixture and incubated at 37°C for 30 min for plasma, 180 min for cardiac tissue, 120 min for renal tissue, 10 min for pulmonary tissue and 60 min for thoracic aorta, respectively. The reaction solution (100 μl) was removed into 0.1 N NaOH solution and 25 μl of 2% *ortho*-phthaldialdehyde was added. Thirty

Table 1 Effects of oral treatment with perindopril or enalapril on systolic blood pressure and heart rate in rats with myocardial infarction

		n	Baseline	Treatment with ACE inhibitors: 1 week	2 weeks	3 weeks	4 weeks
Systolic blood pressure							
Sham		14	96.3 ± 2.8	101.7 ± 3.2*	109.2 ± 3.4*	107.4 ± 3.5*	103.8 ± 3.7*
Control		17	97.4 ± 2.9	81.9 ± 2.0	89.1 ± 2.0	90.9 ± 2.1	91.8 ± 2.2
Perindopril	0.2 mg kg^{-1} day^{-1}	17	97.6 ± 2.5	88.5 ± 2.2	92.0 ± 4.1	93.5 ± 3.6	93.6 ± 3.4
	2 mg kg^{-1} day^{-1}	17	98.8 ± 3.4	84.4 ± 2.7	74.6 ± 2.2*	72.6 ± 2.3*	75.2 ± 3.2*
Enalapril	2 mg kg^{-1} day^{-1}	17	97.3 ± 1.5	85.8 ± 2.0	88.5 ± 3.5	88.6 ± 2.6	89.3 ± 3.3
	20 mg kg^{-1} day^{-1}	17	102.3 ± 3.3	87.9 ± 3.4	80.8 ± 2.3*	82.4 ± 1.7*	78.8 ± 2.7*
Heart rate							
Sham		14	456.0 ± 12.0	410.4 ± 13.0*	417.1 ± 6.9	441.3 ± 13.4	431.3 ± 10.2
Control		17	441.3 ± 12.1	443.4 ± 10.2	416.1 ± 12.0	417.1 ± 12.8	433.1 ± 8.5
Perindopril	0.2 mg kg^{-1} day^{-1}	17	457.1 ± 10.5	456.0 ± 9.8	428.5 ± 10.8	436.3 ± 10.0	424.2 ± 8.4
	2 mg kg^{-1} day^{-1}	17	440.5 ± 8.1	426.1 ± 7.9	426.9 ± 7.2	439.3 ± 7.4	426.2 ± 7.5
Enalapril	2 mg kg^{-1} day^{-1}	17	443.9 ± 7.8	433.9 ± 7.0	429.3 ± 9.1	435.9 ± 7.3	438.8 ± 9.7
	20 mg kg^{-1} day^{-1}	17	420.3 ± 7.0	426.5 ± 9.1	442.7 ± 12.0	427.5 ± 7.2	441.3 ± 7.8

Values are mean ± s.e.mean. *n*, number of animals. *$P < 0.05$ vs. control treatment group.

minutes later, 1 ml of 0.8 N HCl was added and centrifuged at 900 *g* for 10 min. Then, fluorospectrometry of the sample was performed (excitation: 355 nm, emission: 460 nm) with a fluoroscanner (Titertek, Fluoroskan II, type 371, Flow Laboratories Japan Co. Ltd., Tokyo, Japan).

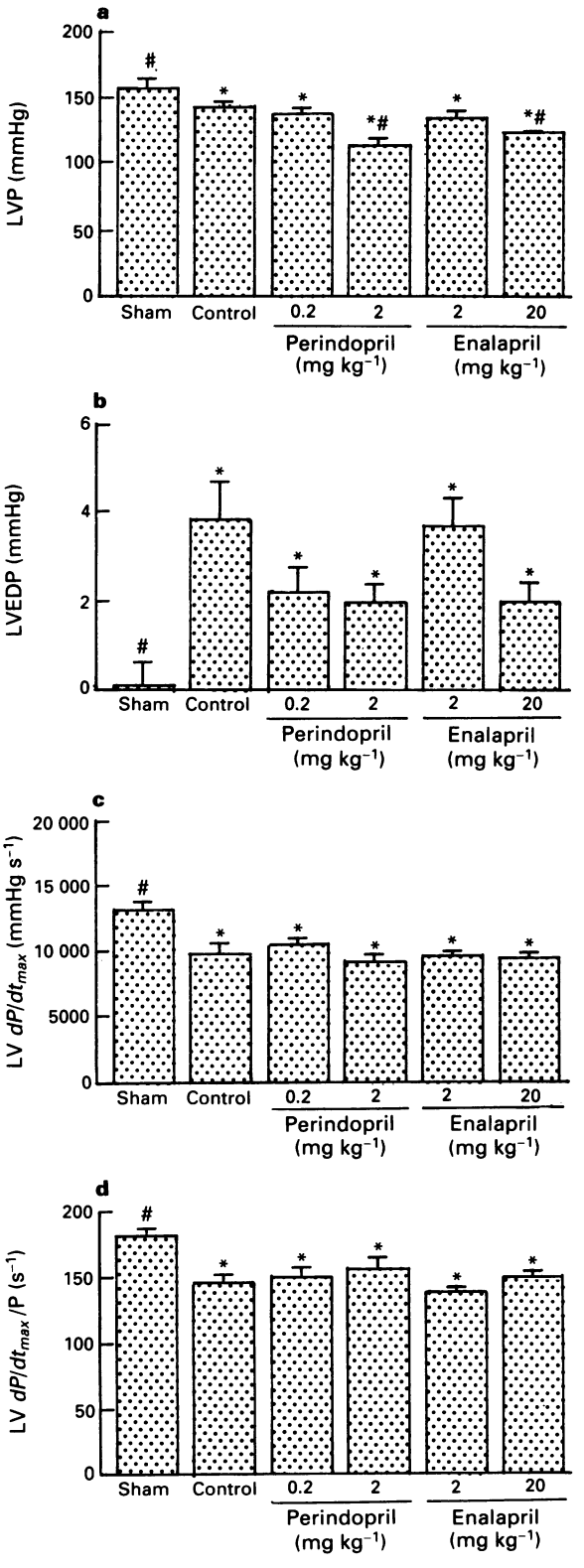

Figure 1 Cardiac function in sham-operated and coronary-ligated rats treated with perindopril (0.2 or 2 mg kg^{-1} day^{-1}), enalapril (2 or 20 mg kg^{-1} day^{-1}), or water (control). (a) LVP, left ventricular pressure; (b) LVEDP, left ventricular end-diastolic pressure; (c) LV dP/dt_{max}, the maximum rate of rise in LVP; (d) LV dP/dt_{max}/P, LV dP/dt_{max} divided by instantaneous LVP. *$P<0.05$ versus sham-operated rats; #$P<0.05$ versus control-treatment rats. Results are expressed as means ± s.e.mean of 18–23 animals.

Drugs

Perindopril tert-butylamin was obtained from Institut De Recherches Internationales Servier (Courbevoie Cedex, France). Enalapril malate was synthesized at Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). Hippuryl-L-histidine-L-leucine and angiotensin I were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.); *ortho*-phthaldialdehyde from Peptide Institute, Inc. (Osaka, Japan); halothane from Takeda Chemical Industries, Ltd. (Osaka, Japan).

Statistical analysis

Results are expressed as mean ± s.e.mean; *n* refers to the number of animals. Statistical comparison was performed by means of Kruskal-Wallis test, followed by Mann-Whitney test. A *P* value of less than 0.05 were considered to indicate statistically significant differences between groups.

Results

Changes in systemic blood pressure and heart rate after coronary ligation (Table 1)

There was no significant difference between the groups in systolic blood pressure and heart rate at the beginning of the experiments. In the coronary-ligated rats, systolic blood pressure decreased one week after the ligation from 98.7 ± 1.2 mmHg to 86.1 ± 1.2 mmHg (in total, $n = 85$). The oral administration of perindopril (2 mg kg^{-1} day^{-1}) and enalapril (20 mg kg^{-1} day^{-1}) reduced systolic blood pressure, which lasted until the end of the experiments. Neither perindopril nor enalapril affected heart rate.

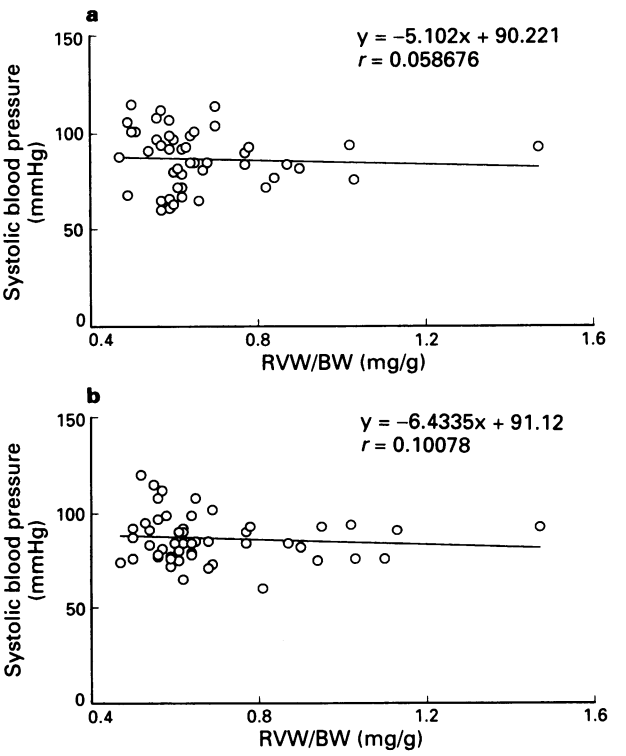

Figure 2 Correlation between cardiac hypertrophy and systemic blood pressure. (a) Controls and perindopril-treated group (0.2 and 2 mg kg^{-1} day^{-1}); (b) controls and enalapril-treated groups (2 and 20 mg kg^{-1} day^{-1}). RVW/BW, a ratio of right ventricular weight to body weight. *r* = correlation coefficient.

Table 2 Effects of oral treatment with perindopril or enalapril on cardiac hypertrophy and infarct size in rats with myocardial infarction

		Coronary ligation				
			Perindopril		*Enalapril*	
	Sham ($n = 14$)	*Control* ($n = 17$)	0.2 mg kg^{-1} day^{-1} ($n = 17$)	2 mg kg^{-1} day^{-1} ($n = 17$)	2 mg kg^{-1} day^{-1} ($n = 17$)	20 mg kg^{-1} day^{-1} ($n = 17$)
Weights						
BW (g)	434 ± 5‡	398 ± 6*	393 ± 6*	381 ± 7*	400 ± 6*	380 ± 7*‡
RVW (mg)	249 ± 7	301 ± 19	240 ± 9	229 ± 7	277 ± 20	228 ± 7*‡
LVW (mg)	846 ± 18	801 ± 16	786 ± 19*‡	678 ± 10*‡	757 ± 10*‡	707 ± 11*‡
WVW (mg)	1095 ± 24	1102 ± 19	1026 ± 25‡	906 ± 15*‡	1034 ± 24*‡	935 ± 14*‡
Cardiac hypertrophic indices						
RVW/BW (mg/g)	0.57 ± 0.01‡	0.76 ± 0.06*	0.61 ± 0.02‡	0.60 ± 0.02‡	0.69 ± 0.05	0.60 ± 0.02‡
LVW/BW (mg/g)	1.95 ± 0.03	2.02 ± 4.04	2.00 ± 0.04	1.78 ± 0.02	1.90 ± 0.02‡	1.87 ± 0.04‡
WVW/BW (mg/g)	2.52 ± 0.04‡	2.78 ± 0.07*	2.61 ± 0.05	2.38 ± 0.03*‡	2.59 ± 0.06‡	2.47 ± 0.04‡
Infarction size (%)		56 ± 3	49 ± 3	45 ± 3‡	44 ± 2‡	43 ± 3‡

Values are mean ± s.e.mean. BW, body weight; RVW, right ventricular weight; LVW, left ventricular weight; WVW, whole ventricular weight; infarction size, percent of the ventricle; n, number of animals. *$P < 0.05$ vs. sham; ‡$P < 0.05$ vs. control.

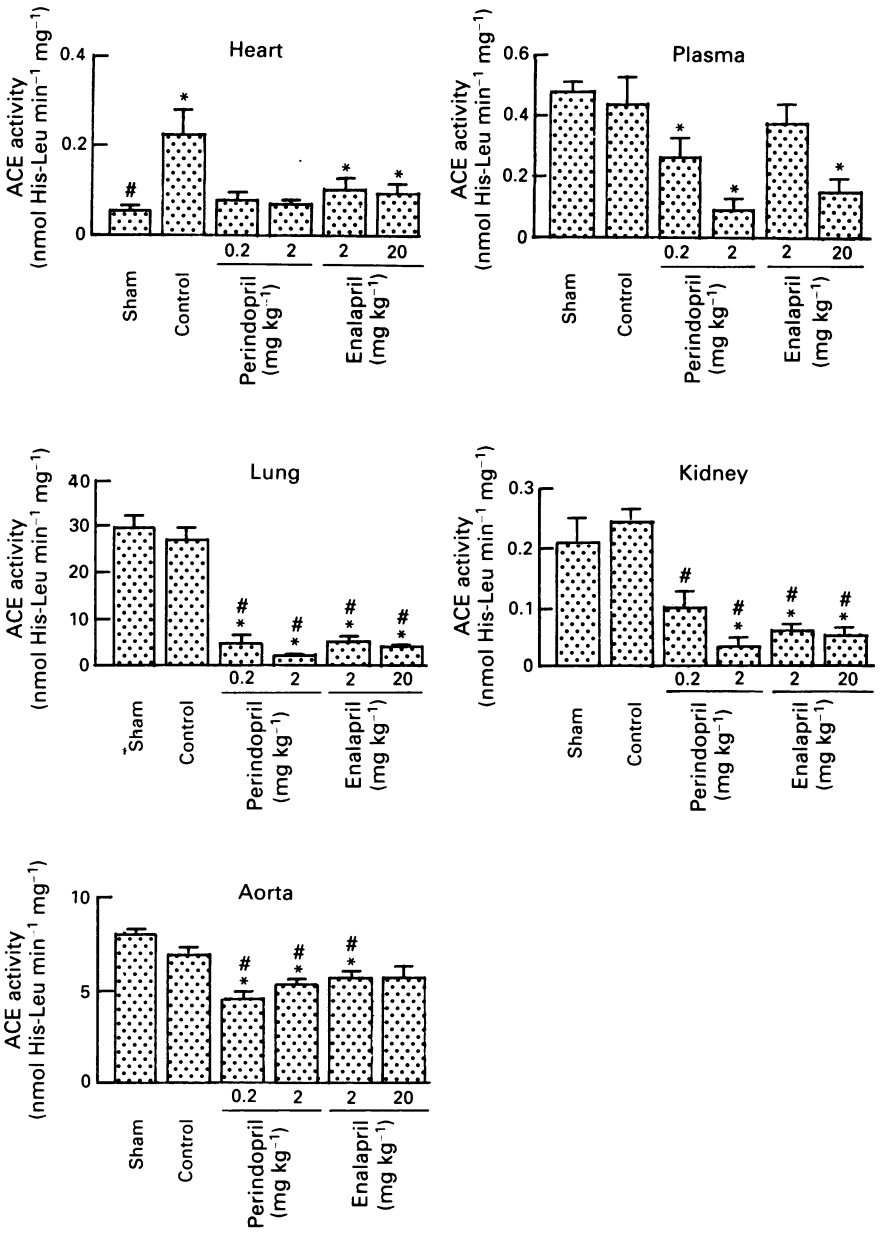

Figure 3 Angiotensin converting enzyme (ACE) activity in sham-operated and coronary-ligated rats treated with perindopril (0.2 or 2 mg kg^{-1} day^{-1}), enalapril (2 or 20 mg kg^{-1} day^{-1}), or water (control). *$P < 0.05$ versus sham-operated rats; #$P < 0.05$ versus control rats. Results are expressed as means ± s.e.mean of five animals.

Cardiac function at the end of treatment (Figure 1)

In the controls, left ventricular pressure (LVP), the maximum rate of rise of LVP (LV dP/dt_{max}) and LV dp/dt_{max} divided by instantaneous LVP (LV $dP/dt_{max}/P$; an index of cardiac contractility) were reduced, and left ventricular end-diastolic pressure (LVEDP) was increased compared to the sham-operated group. Perindopril (2 mg kg^{-1} day^{-1}) and enalapril (20 mg kg^{-1} day^{-1}) decreased LVP and LV dP/dt_{max}, respectively. There was no significant difference in LVEDP and LV $dp/dt_{max}/P$ between the controls, perindopril- and enalapril-treated groups.

Cardiac weight and myocardial infarction size (Table 2)

The ratio of right ventricular weight to body weight (RVW/BW) was higher in the controls than in the sham-operated group. This increase in RVW/BW was significantly attenuated in the animals treated with perindopril (0.2 mg kg^{-1} day^{-1} or 2 mg kg^{-1} day^{-1}) or enalapril (20 mg kg^{-1} day^{-1}), but not in the animals treated with 2 mg kg^{-1} day^{-1} enalapril. There was no significant correlation between RVW/BW and systemic blood pressure at the end of treatments with perindopril ($r = 0.06$) or enalapril ($r = 0.1$) (Figure 2). Although myocardial infarction size varied in a narrow range (43.0–56.2% of left ventricle) in all of the coronary-ligated rats, the size in animals treated with perindopril (2 mg kg^{-1} day^{-1}) and enalapril (2 and 20 mg kg^{-1} day^{-1}) was significantly smaller than in controls.

ACE activity (Figure 3)

Cardiac ACE activity was four fold higher in the control treatment group with coronary ligation (control) than in the sham-operated group. In contrast, there was no significant difference between control and sham-operated groups in ACE activities in plasma and kidney, lung and aorta. The increase in cardiac ACE activity was significantly inhibited by chronic treatment with perindopril (0.2 and 2 mg kg^{-1} day^{-1}), but not by treatment with enalapril (2 and 20 mg kg^{-1} day^{-1}). ACE activities in the kidney and lung were lowered in the groups treated with perindopril or enalapril at both doses, respectively. Aortic ACE activity was suppressed in the perindopril-treated (0.2 and 2 mg kg^{-1} day^{-1}) and 2 mg kg^{-1} day^{-1} enalapril-treated groups, but not in the 20 mg kg^{-1} day^{-1} enalapril-treated group.

Discussion

To clarify the beneficial effects of chronic treatment with ACE inhibitors on cardiac hypertrophy and dysfunction after coronary ligation, the present study focused on the changes in haemodynamics and tissue ACE activities. In the acute phase of myocardial infarction, the activation of renin in plasma can alter systemic haemodynamics, which may affect the chronic progression of cardiac hypertrophy and dysfunction (Dzau *et al.*, 1981). To avoid an interaction with the acute effects of ACE inhibitors on haemodynamics, the inhibitors were given to the rats from one week after ligation of coronary arteries, when the activities of circulating renin-angiotensin system return to baseline. The selection of two oral doses of each perindopril or enalapril was based on the effects of the compounds on systemic blood pressure in preliminary studies (data not shown); lower doses which did not alter blood pressure and higher doses which decreased systolic blood pressure by 10–15 mmHg.

At the end of the experiments, four weeks after coronary ligation, significant cardiac hypertrophies were induced in the right ventricles. The progression of right ventricular hypertrophy is an adaptive response to the increased pre- and afterload due to impaired function of left ventricle with myocardial infarction. The hypertrophy in the right ventricle was associated with the elevation of LVEDP and increase in cardiac ACE activity in the controls with coronary ligation compared to sham-operated animals. However, ACE activities were not enhanced in plasma, lung, kidney and aorta, indicating a selective activation of cardiac ACE in rats with myocardial infarction. The present results are consistent with previous findings (Hirsch *et al.*, 1991). Chronic treatment with perindopril at both doses of 0.2 and 2 mg kg^{-1} day^{-1} attenuated right ventricular hypertrophy and lowered ACE activities in heart, lung, aorta and kidney, but not in plasma. Perindopril at 0.2 mg kg^{-1} day^{-1} did not alter systemic blood pressure and heart rate during the experiments. The increase in LVEDP in controls may contribute to stimulate the progression of the cardiac hypertrophy. However, perindopril and enalapril did not significantly reduce LVEDP at the end of experiments. In contrast to perindopril, enalapril at non-hypotensive dose (2 mg kg^{-1} day^{-1}) did not inhibit right ventricular hypertrophy. A dose of 20 mg kg^{-1} day^{-1} of enalapril was required (which lowered blood pressure) to attenuate the hypertrophy in right ventricles. However, it is unlikely that enalapril inhibits cardiac hypertrophy due to its effects on haemodynamics since there is no correlation between systolic blood pressure and right ventricular hypertrophy at the end of treatment with enalapril. Hence, the present findings demonstrate that the beneficial effects of ACE inhibitors are independent of a significant fall in blood pressure on the cardiac hypertrophy. Thus, the direct influence on haemodynamics is separate from the anti-hypertrophic effects of ACE inhibitors in the rat heart failure model of coronary ligation. The present results are in agreement with previous studies showing that ramipril prevents left ventricular hypertrophy without blood pressure reduction in the aortic stenosis model in rats (Linz *et al.*, 1989; Linz & Schölkens, 1992). Alternatively, the present findings suggest that selective augmentation of ACE activities in cardiac tissues explains, at least in part, the progression of cardiac hypertrophy after coronary ligation. Stimulation of conversion of angiotensin I to angiotensin II may lead to cell growth of myocytes. The discrepancy between the two ACE inhibitors, perindopril and enalapril, in the selectivity of actions on cardiac hypertrophy and haemodynamics may be due to the different potency of ACE inhibition in hearts and vessels (Hirsch *et al.*, 1992). The attenuation by perindopril of the progression of cardiac hypertrophy and the increase in cardiac ACE activity is consistent with earlier observations (Pfeffer *et al.*, 1985; 1988; Hirsch *et al.*, 1991; Fornes *et al.*, 1992). Perindopril (2 mg kg^{-1} day^{-1}) and enalapril (2 and 20 mg kg^{-1} day^{-1}) lowered the weight of left ventricles including septum (LVW or LVW/BW). The inhibitors may suppress the cell growth in the non-infarcted region of the left ventricle; however, the interpretation of the present results requires caution since most of the infarcted myocardium had degenerated. The reduction of infarct size by perindopril and enalapril might attenuate the impaired cardiac function.

In addition, the results of the present study do not rule out other possibilities concerning the mechanism of action of ACE inhibitors. The enhanced concentration of bradykinin produced by ACE inhibitors in the hearts may participate in the regulation of cardiac hypertrophy since the anti-hypertrophy effect of ramipril is inhibited by Hoe 140, a kinin B_2 receptor antagonist (Linz & Schölkens, 1992). Furthermore, angiotensin II stimulates the release of noradrenaline from adrenergic nerve endings, which may accelerate cardiac hypertrophy due to the activation of α_1-adrenoceptors (Newling *et al.*, 1989).

In conclusion, the present study demonstrates a separation of hypotensive effects from anti-hypertrophic actions of ACE inhibitors after myocardial infarction in rats.

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