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Force–length relationship in dogs as a measure of protective effect of imidapril on regional myocardial ischemia and reperfusion injury

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Abstract

Our laboratory previously reported that the end-systolic force–length relationship of the left ventricle provides a better method of evaluating myocardial contractile properties than the left ventricular end-systolic pressure–volume relationship, because it avoids deficiencies of the latter parameter such as dependence of its slope (E_{max}) on the volume intercept (V_0). The slope (E_c) of the left ventricular end-systolic force–length relationship represents the contractility of functioning myocardium, while its length intercept (L_0) reflects the length of non-functioning myocardium. However, the effect of regional myocardial ischemia on these parameters, as evaluated by the force–length relationship, remains unknown. To clarify the effects of regional ischemia and angiotensin-converting enzyme inhibition on the myocardium during ischemia–reperfusion, the changes in E_c and L_0 were determined in anesthetized open-chest dogs. (1) Control group ($n = 26$): Before and after 15 min of complete coronary artery occlusion, as well as after 15 min of reperfusion, left ventricular pressure and volume were simultaneously recorded during inferior vena cava occlusion. The left ventricular force–length relationship was obtained from the pressure and volume of three cylindrical segments of the ventricle, and E_c and L_0 were calculated. (2) Imidapril group ($n = 14$): Imidaprilat (1 μg/kg/min) was continuously infused from 30 min before ischemia to the end of the experiment, and the same procedures were followed as in the control group. Fourteen out of the 26 dogs (54%) in the control group died of reperfusion-induced ventricular arrhythmias, while only two of the 14 dogs (14%) in the imidapril group did so ($P < 0.05$). In the control group, E_c was increased during ischemia and remained at the same level after reperfusion. However, E_c was not altered in the imidapril group. Although L_0 was increased during ischemia and decreased after reperfusion in both groups, the percent increase of L_0 in the imidapril group was significantly smaller than in the control group (8% vs. 32%, $P < 0.05$). With the improvement of these indices, the bradykinin concentration of coronary venous blood increased in the imidapril group ($P < 0.01$). These findings suggest that regional myocardial ischemia increased the average contractility of overall functioning myocardium despite the increased non-functioning myocardium. Moreover, imidapril has a cardioprotective effect against ischemia–reperfusion injury by decreasing infarct size, and through the antiarrhythmic effect and the reversal of increased overall contractility.

Keywords: Angiotensin-converting enzyme inhibitor; Bradykinin; Ischemia; Reperfusion; Ventricular function, dog

1. Introduction

The end-systolic force–length relationship of the left ventricle determined from the active cross-bridge model (Takeda, 1990; Takeda et al., 1991a, 1993) and the cylinder model (Takeda et al., 1988, 1990, 1991b) seems to provide a better method of evaluating myocardial contractile properties than the left ventricular end-systolic pressure–volume relationship, because it avoids deficiencies of the latter parameter such as the dependence of its slope (E_{max}) on the volume intercept (V_0). The slope (E_c) of the left ventricular end-systolic force–length relationship represents the contractility of functioning myocardium, while its length intercept (L_0) reflects the length of non-functioning myocardium. However, the effect of regional myocardial ischemia on these parameters derived from the force–length relationship remains unknown. Many reports have been published indicating that angiotensin-converting enzyme inhibitors have a cardioprotective effect and can reduce the extent of infarction after ischemia and reperfusion (Ertl et al., 1982, 1983; Lefer and Peck, 1984; Hock et al., 1985; De Graeff et al., 1987, 1988; Martorana et al., 1990; Noda et al., 1993; Hartmann et al., 1993; Node et

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al., 1998). However, the effect of angiotensin-converting enzyme inhibitor on these parameters is also not fully understood.

In the present study, therefore, we used E_c and L_0 to evaluate changes in myocardial contractile properties during regional myocardial ischemia and after reperfusion. In addition, the changes in E_c and L_0 were also determined during intravenous infusion of an angiotensin-converting enzyme inhibitor to clarify the acute effect of this drug class on the myocardium during ischemia and after reperfusion.

2. Materials and methods

2.1. Animal model

Forty-five healthy adult mongrel dogs (11.8 ± 1.2 kg) were used in this study. They were anesthetized with intravenous sodium pentobarbital (26 mg/kg) and instrumented as described elsewhere (Takeda et al., 1990). In brief, positive pressure ventilation was provided via an endotracheal tube. Thoracotomy was performed at the left fourth intercostal space, the pericardium was opened, and the heart was suspended in a pericardial cradle. A micromanometer-tipped catheter (MPC500, Millar Instruments, USA) was balanced in a constant-temperature water bath (37°C), after which it was inserted into the carotid artery and advanced to the left ventricle. A 5F Swan–Ganz catheter was inserted into the right femoral vein and was advanced to the pulmonary artery for the infusion of drugs and fluid and for measurement of the cardiac output. Another catheter was inserted into the right femoral artery for blood gas analysis. An occluder was set around the inferior vena cava to produce a decrease in preload. A conductance catheter was inserted through the left ventricular apex. The stability of the left ventricular pressure–volume loops obtained for three cylindrical ventricular segments [$V_2(t)$, $V_3(t)$, and $V_4(t)$] was tested with a computer monitor during both steady state and preload manipulation. The following five variables were measured: left ventricular pressure, lead(II) of the surface electrocardiogram, and the cylindrical volume of left ventricular segments 2, 3, and 4. The data for these variables were simultaneously stored in the hard disk memory of a computer (PC9801VX21, NEC, Japan) at 1-ms intervals, and were subsequently evaluated without filtering. The present investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Conductance measurement

A full description of the Baan volume catheter method is described elsewhere (Baan et al., 1981, 1984; Burkhoff et al., 1985). Briefly, the conductance method of determining left ventricular volume is based on measuring the conductivity of the blood inside the left ventricular cavity. The catheter used had eight electrodes located distal to the tip with a constant inter-electrode distance of 0.9 (or 0.75) cm. This catheter was connected to a conditioning amplifier (model SIGMA-5, LEYCOM, Netherlands), which passed a 20 kHz and 30 μA current between the most distal and proximal electrodes. The intervening electrodes (2nd–7th) were used to form five successive voltages that were assumed to correspond to five cylindrical intraventricular segments with an equal height (L). These voltages were proportional to the conductance of each segment, so the sum of the five segmental conductances [$G_1(t)$, $G_2(t)$, $G_3(t)$, $G_4(t)$, and $G_5(t)$] was considered to represent the left ventricular volume. However, the conductance of two of the cylinders [$G_1(t)$ and $G_5(t)$] was ignored in the present experiment. The following equation was used:

$$V_i(t) = (1/\alpha)(L^2/\sigma)G_i(t) - V_{pi} \quad (1)$$

where σ is the conductivity of blood around the catheter in the ventricular cavity. In addition, V_{pi} (V_{p2}, V_{p3}, or V_{p4}) is a volume signal error due to conduction of current through the left ventricular wall and other tissues, which can be estimated by the bolus injection of hypertonic saline into the pulmonary artery. Alpha (α) is an empirical slope coefficient for the $V_i(t)$–$G_i(t)$ relationship, which was previously determined by comparison with the thermodilution and flow output in an isolated heart preparation (Baan et al., 1984), and was found to vary between 0.85 and 1.1. Measurements obtained in several closed-chest dogs, using the thermodilution cardiac output, yielded a mean value of 1.0. In the present experiments, we did not determine the slope coefficient for each animal, but instead used this mean value 1.0 (Kass et al., 1986). An analog computer/stimulator (model SIGMA-5, LEYCOM) was used to provide the current source and to process the segmental conductances, yielding a continuous instantaneous analog volume signal.

2.3. Experimental protocol

β-Adrenoceptor and vagal blockade was obtained with intravenous propranolol (2 mg/kg) and atropine (0.2 mg/kg), respectively (Vatner et al., 1972; Little et al., 1985). A major reflex change of autonomic tone was defined as an increase or decrease in heart rate of more than 10 beats/min over the course of caval occlusion (Little et al., 1985). Arterial blood gases were determined with an analyzer (model ABL4, Radiometer, Denmark) while the dogs were being ventilated (arterial p_aO_2 and p_aCO_2 were > 100 and < 40 mm Hg, respectively).

Control group ($n = 26$): When steady state was achieved at least 10 min after the initiation of β-adrenoceptor and vagal blockade, the inferior vena cava was gradually oc-

cluded while data on the left ventricular cylinder volumes, left ventricular pressure, and electrocardiogram were collected (arrow 1 in Fig. 1). To eliminate the effect of changes in lung volume due to respiration, all data were recorded during 20-s periods of apnea. Cardiac output (EH-11 cardiac output computer, Fukuda Denshi, Japan) was measured by the thermodilution method using the Swan–Ganz catheter. The proximal portion of the left anterior descending coronary artery was completely occluded for 15 min to produce regional myocardial ischemia, and then data recording was repeated (arrow 2 in Fig. 1). The development of regional ischemia was confirmed at the time of coronary occlusion by inspection of the left ventricular wall for cyanosis and akinesia. After data recording during ischemia, the coronary artery occluder was removed and 15 min of reperfusion was allowed before data recording was performed again (arrow 3 in Fig. 1). Finally, to estimate the volume signal error due to conduction of the alternating current through the left ventricular wall and other tissues, 2 ml of 5 N NaCl was injected into the pulmonary artery.

Imidapril group ($n = 14$): Imidaprilat (1 μg/kg/min) was continuously infused into the femoral vein throughout the experiment. At 30 min after the initiation of imidaprilat infusion, the same procedures were performed as in the control group and then data were recorded (Fig. 1).

Non-ischemic imidapril group ($n = 5$): No ischemia was induced in this group and the same measurements were obtained 30, 45, and 60 min after the start of the imidaprilat infusion (Fig. 1).

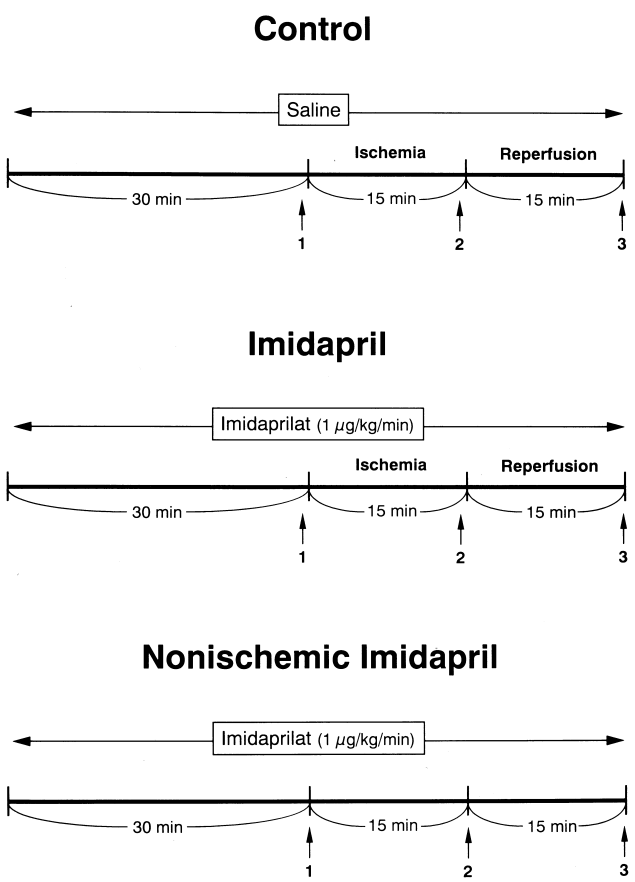

Fig. 1. Outline of the experimental protocol. Data were recorded at points 1, 2, and 3 (indicated by arrows).

In the preliminary experiment, five doses of imidaprilat (0.1, 0.5, 1, 5, and 10 μg/kg/min) were tested to find the maximum dose that did not significantly affect the basal hemodynamic parameters such as blood pressure. Angiotensin-converting enzyme inhibition was assessed from the response of the mean arterial pressure to intravenous infusion of angiotensin I at 50 pmol/kg (Ikeo et al., 1992). In the groups treated with 1, 5, and 10 μg/kg/min imidaprilat, the pressor response to angiotensin I was markedly inhibited, while it was not inhibited in the groups treated with 0.1 and 0.5 μg/kg/min imidaprilat. In the groups treated with 5 and 10 μg/kg/min imidaprilat, the blood pressure showed a significant decrease compared with the baseline value. Therefore, the dose of 1 μg/kg/min imidaprilat was employed in the present study.

Coronary venous blood was withdrawn from the coronary sinus 30 min before coronary artery occlusion, after occlusion, before and after reperfusion, and after 15 and 30 min of reperfusion. Assays were conducted for bradykinin, nitrate (NO_3^-), and nitrite (NO_2^-). Bradykinin was determined by radioimmunoassay (Mashford and Roberts, 1972), while nitrate and nitrite were analyzed by an automated procedure based on the Griess reaction (Green et al., 1982).

None of the dogs developed arrhythmias during preload manipulation and the micromanometer drift was less than 1.0 mm Hg.

2.4. Theoretical background

Instantaneous left ventricular pressure (p; mm Hg) and myocardial length (L; cm) data recorded from several cardiac cycles during the manipulation of preload were used for the construction of left ventricular force–length loops. Left ventricular circumferential force (F, g) was calculated from the equation $F = pL(l/2a\pi)$, where l is the height of a cylinder (1 cm) and a is a conversion factor of 0.735 mm Hg g^{-1} cm^2 (Takeda, 1990). The circumferential myocardial length (L) for the cylindrical segment was estimated from the equation $L = 2(\pi V/d)^{1/2}$, where V (ml) is the overall volume of the three left ventricular cylinders obtained using the conductance catheter and d (cm) is the inter-electrode distance of the conductance catheter (0.9 or 0.75 cm).

End-systole was defined as the upper left corner of the left ventricular force–length loop, i.e., the point where the ratio of myocardial force to length $[F/(L - L_0)]$ was maximal (Takeda et al., 1988). The initiation of systole (end-diastole) was defined as corresponding to the R wave

Table 1
Effect of myocardial ischemia and reperfusion in the control group
Data are presented as mean ± S.D.

	Before	Ischemia	Reperfusion	F value
Heart rate (beats/min)	109.3 ± 15.1	108.1 ± 15.0	109.4 ± 15.3	0.50
Left ventricular pressure (mm Hg)				
End-diastolic	7.3 ± 1.9	9.4 ± 0.6	7.1 ± 1.9[a]	3.95[b]
End-systolic	113.5 ± 13.4	110.3 ± 12.3	112.4 ± 13.2	0.54
Left ventricular volume (ml)				
End-diastolic	21.2 ± 6.8	25.9 ± 11.2[c]	23.9 ± 11.8	3.46[b]
End-systolic	13.0 ± 6.1	18.3 ± 9.2[d]	16.2 ± 9.6[a]	8.11[e]
Left ventricular ejection fraction	0.41 ± 0.14	0.30 ± 0.16[d]	0.34 ± 0.15[a]	8.37[e]
Cardiac output (l/min)	1.56 ± 0.40	1.59 ± 0.46	1.53 ± 0.44	0.57
E_c (g/cm)	44.5 ± 10.7	53.6 ± 10.7[c]	51.0 ± 8.9	4.92[b]
L_0 (cm)	6.12 ± 2.66	9.04 ± 2.77[d]	8.05 ± 3.03[a]	9.81[e]
K_a (1/s)	14.9 ± 2.2	14.7 ± 2.9	14.7 ± 1.8	0.13

[a] $P < 0.05$ vs. ischemia.
[b] $P < 0.05$ for the F value.
[c] $P < 0.05$ vs. before ischemia.
[d] $P < 0.01$ vs. before ischemia.
[e] $P < 0.01$ for the F value.

on the surface electrocardiogram. Left ventricular end-systolic circumferential force (F_{es}) was assumed to be linearly proportional to myocardial length. Thus, F_{es} was expressed by the following equation (Takeda, 1990):

$$F_{es} = E_c(L_{es} - L_0) \quad (2)$$

where E_c (g/cm) denotes the slope of the left ventricular end-systolic force–length relation (an index of the inotropic state of the left ventricular myocardium) and L_0 (cm) is the basal myocardial length. End-systolic force (F_{es}) and length (L_{es}) data were used to obtain the values of E_c and L_0. The ejection fraction was calculated from the overall left ventricular cylinder volume obtained at end-systole and end-diastole. The total contraction period (T_{sys}) of the left ventricle was defined as the time from the initiation of systole to end-systole at steady state, and K_a (1/s) was estimated as follows (Takeda et al., 1993):

$$K_a = 3/T_{sys} \quad (3)$$

Our previous studies (Takeda, 1990; Takeda and Yagi, 1991; Takeda et al., 1988, 1990, 1991a,b, 1993) have suggested that E_c is an appropriate index of the contractility of the functioning left ventricular myocardium (Hooke's law; Atkins, 1970), that L_0 may provide a measure of the length of non-functioning myocardium, and that K_a is a potentially useful index for evaluating adrenergic activity related to the working left ventricular myocardium.

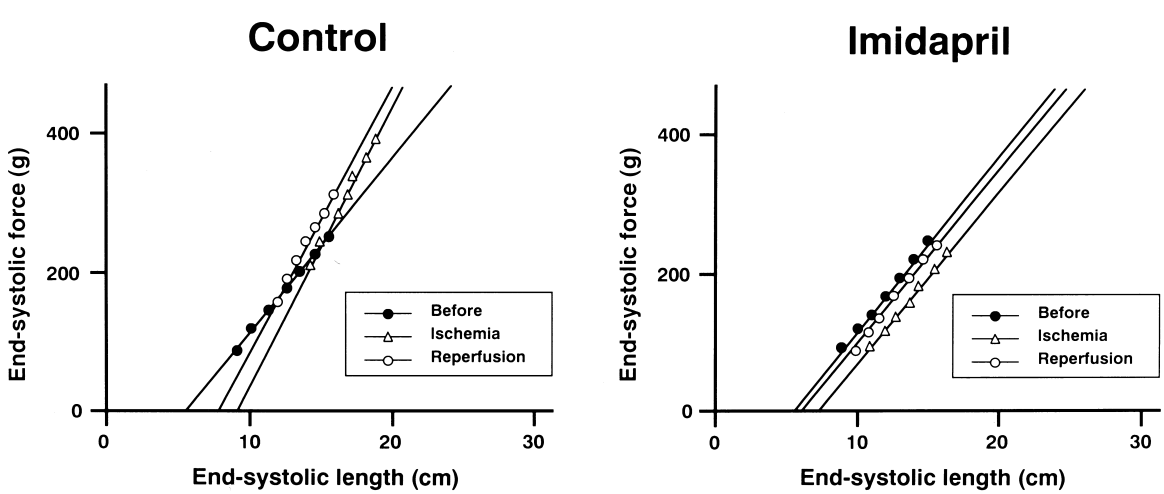

Fig. 2. Changes in the slope of the left ventricular force–length relation (E_c) with ischemia and reperfusion. In the control group, E_c was significantly increased during ischemia and remained around the same level after reperfusion. In contrast, E_c was not altered in the imidapril group.

2.5. Data and statistical analysis

The digital data stored on the hard disk were processed using a computer (PC9801VX21, NEC, Japan) and software developed at our laboratory. Results are expressed as mean ± S.D. The statistical significance of differences between mean values was assessed by repeated measures of analysis of variance (ANOVA). To assess the significance of differences in the bradykinin and nitric oxide concentrations or in the percent increase of E_c and L_0, the Mann–Whitney *U*-test and the Wilcoxon signed-rank test were employed, respectively. The chi-square test was performed to compare the mortality rate due to reperfusion-induced ventricular arrhythmias. Linear regression analysis by the least-squares method was used to fit data to the left ventricular F_{es}–L_{es} relationship. A level of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Animal loss

Fourteen of the 26 dogs (54%) in the control group died of reperfusion-induced ventricular arrhythmias, while only two of the 14 dogs (14%) in the imidapril group died ($P < 0.05$). Data could be analyzed for 12 dogs in the control group, 12 dogs in the imidapril group, and five dogs in the non-ischemic imidapril group.

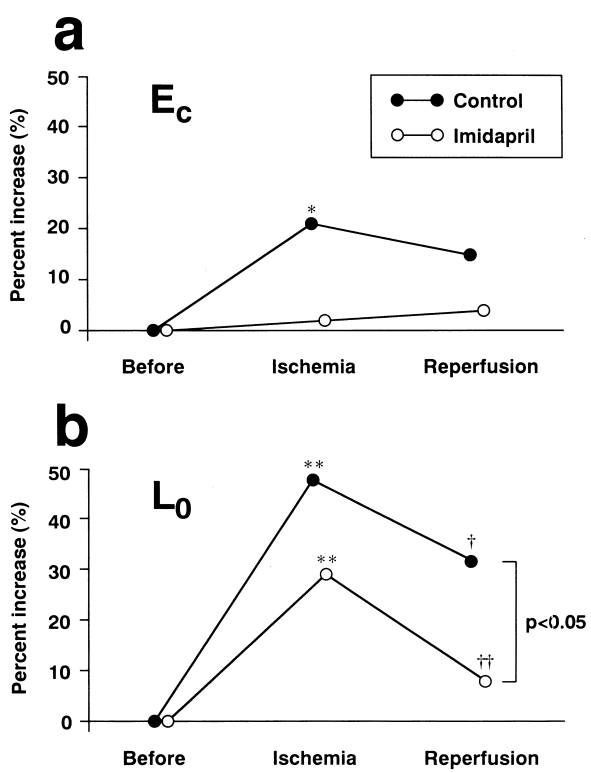

Fig. 3. Percent change of E_c (a) and L_0 (b) during ischemia and reperfusion. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. before ischemia. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ vs. ischemia.

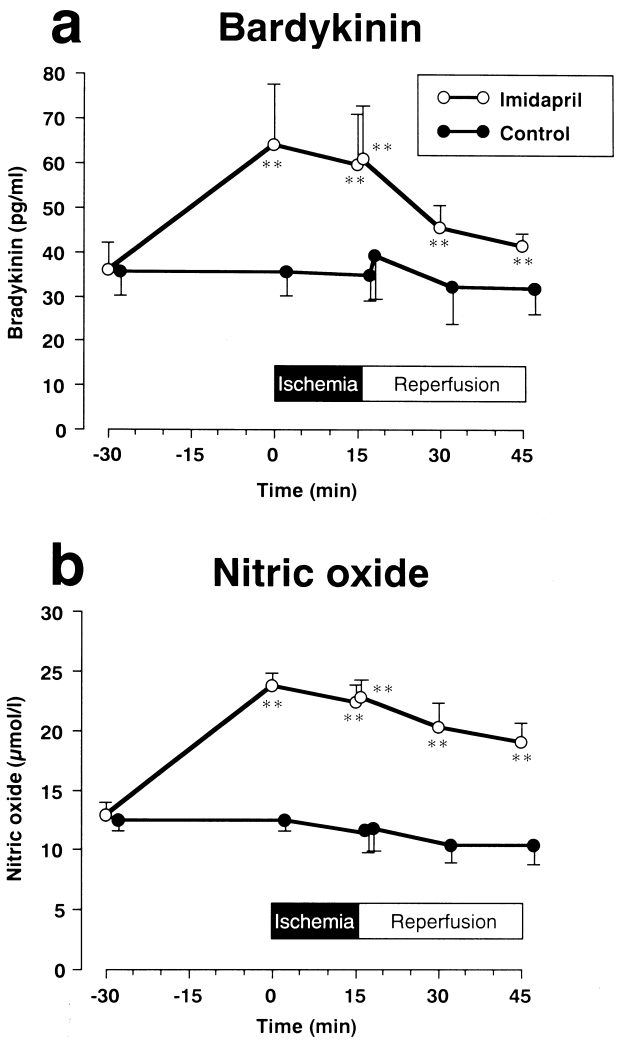

Fig. 4. Changes in the bradykinin (a) and nitric oxide (b) levels in coronary venous blood during ischemia and reperfusion. Values are expressed as mean ± S.D. $^{**}P < 0.01$ vs. control group.

3.2. Hemodynamic data during regional ischemia and after reperfusion

Control group (Table 1): The heart rate, left ventricular end-diastolic pressure, and left ventricular end-systolic pressure were not changed by ischemia, but the left ventricular end-diastolic volume and left ventricular end-systolic volume were significantly increased. In contrast, the left ventricular ejection fraction was significantly decreased by regional myocardial ischemia. However, the change in cardiac output produced by ischemia was not statistically significant. The values of E_c and L_0 estimated from the overall volume of the three left ventricular cylindrical segments were significantly increased by regional ischemia (Figs. 2 and 3), whereas the cross-bridge activa-

Table 2
Effect of myocardial ischemia and reperfusion in the imidapril group
Data are presented as mean ± S.D.

	Before	Ischemia	Reperfusion	F value
Heart rate (beats/min)	111.9 ± 17.2	112.9 ± 17.3	113.2 ± 19.2	1.41
Left ventricular pressure (mm Hg)				
End-diastolic	7.5 ± 2.5	9.0 ± 2.6[a]	7.0 ± 2.7[b]	32.28[c]
End-systolic	119.5 ± 12.7	110.4 ± 13.0[a]	114.8 ± 12.3[b]	12.45[c]
Left ventricular volume (ml)				
End-diastolic	20.8 ± 6.0	22.8 ± 6.4[d]	21.7 ± 7.4[e]	4.43[f]
End-systolic	13.0 ± 5.1	15.8 ± 5.5[d]	14.2 ± 5.8[b]	7.55[c]
Left ventricular ejection fraction	0.38 ± 0.10	0.31 ± 0.09[d]	0.35 ± 0.08[e]	7.85[c]
Cardiac output (l/min)	1.58 ± 0.39	1.57 ± 0.40	1.72 ± 0.35[e]	5.98[c]
E_c (g/cm)	42.3 ± 12.6	43.0 ± 6.4	43.9 ± 10.7	0.27
L_0 (cm)	5.66 ± 2.74	7.29 ± 2.58[a]	6.14 ± 2.71[b]	14.87[c]
K_a (1/s)	14.9 ± 2.4	14.7 ± 2.4	14.9 ± 3.6	0.22

[a] $P < 0.01$ vs. before ischemia.
[b] $P < 0.01$ vs. ischemia.
[c] $P < 0.01$ for the F value.
[d] $P < 0.05$ vs. before ischemia.
[e] $P < 0.05$ vs. ischemia.
[f] $P < 0.05$ for the F value.

tion rate constant (K_a) of the left ventricular myocardium was not altered. After 15 min of reperfusion, the heart rate, left ventricular end-systolic pressure, left ventricular end-diastolic volume, and cardiac output were not changed, but the left ventricular end-diastolic pressure and left ventricular end-systolic volume were decreased, and the left ventricular ejection fraction was increased significantly. Although E_c and K_a were not significantly altered compared with the ischemic state, L_0 was significantly decreased compared to that during ischemia (Figs. 2 and 3). The concentrations of bradykinin and nitric oxide (nitrate plus nitrite) in coronary venous blood were not significantly altered (Fig. 4).

Imidapril group (Table 2): After 15 min of ischemia, left ventricular end-systolic pressure and left ventricular ejection fraction were significantly decreased, while the left ventricular end-diastolic pressure, left ventricular end-diastolic volume, and left ventricular end-systolic volume were significantly increased. Although E_c and K_a were not altered, L_0 was significantly increased (Figs. 2 and 3). The percent increase of L_0 was smaller than that in the control group, but the difference was not significant (29% vs. 48%, NS, Fig. 3b). After reperfusion, the left ventricular end-diastolic pressure, left ventricular end-diastolic volume, and left ventricular end-systolic volume showed a significant decrease, while the end-systolic pressure, left ventricular ejection fraction, and cardiac output were significantly increased. Although E_c and K_a were not significantly altered compared with the ischemic state, L_0 showed a significant decrease compared to that during ischemia (Figs. 2 and 3). The percent increase of L_0 vs. the baseline state was significantly smaller than in the control group

Table 3
Data for the non-ischemic imidapril group
Data are presented as mean ± S.D.

	30 min	45 min	60 min	F value
Heart rate (beats/min)	110.8 ± 15.6	110.2 ± 14.9	110.4 ± 15.3	0.08
Left ventricular pressure (mm Hg)				
End-diastolic	8.0 ± 1.6	7.1 ± 2.2	6.9 ± 2.6	3.22
End-systolic	118.2 ± 16.2	113.0 ± 15.3	109.6 ± 15.8	5.20[a]
Left ventricular volume (ml)				
End-diastolic	20.3 ± 8.7	18.7 ± 7.4	18.8 ± 7.4	1.81
End-systolic	12.3 ± 6.6	11.3 ± 6.0	11.4 ± 5.8	1.57
Left ventricular ejection fraction	0.42 ± 0.11	0.42 ± 0.12	0.42 ± 0.10	0.01
Cardiac output (l/min)	1.53 ± 0.53	1.67 ± 0.66	1.72 ± 0.83	1.63
E_c (g/cm)	40.3 ± 8.1	39.8 ± 9.3	39.8 ± 8.9	0.03
L_0 (cm)	4.98 ± 1.84	4.81 ± 1.92	5.01 ± 1.37	0.35
K_a (1/s)	14.7 ± 1.5	15.0 ± 1.0	14.3 ± 1.0	2.82

[a] $P < 0.05$ for the F value.

(8% vs. 32%, $P < 0.05$, Fig. 3b). The concentrations of bradykinin and nitric oxide in coronary venous blood were significantly higher than in the control group throughout the experiment (Fig. 4).

Non-ischemic imidapril group (Table 3): At 30, 45, and 60 min after starting imidaprilat infusion, the E_c values were 40.3 ± 8.1, 39.8 ± 9.3, and 39.8 ± 8.9 g/cm, respectively, while the L_0 values were 4.98 ± 1.84, 4.81 ± 1.92, and 5.01 ± 1.37 cm, respectively. There were no significant differences between any of these values. K_a also showed no significant changes.

4. Discussion

4.1. Force–length–time relation during regional ischemia

Regional ischemia increased both the left ventricular end-systolic and end-diastolic volumes and significantly decreased the left ventricular ejection fraction, while the cardiac output remained unchanged. The left ventricular non-functioning myocardial length (L_0) and myocardial contractility (E_c) were also increased by regional ischemia. Since regional ischemia was the only intervention directly affecting the left ventricular myocardium in the present model, and ischemia causes a rapid reduction of myocardial force and shortening (Theroux et al., 1974, 1976; Heyndrickx et al., 1975; Ross and Franklin, 1976), it is reasonable to conclude that non-contractile myocardium was produced by 15 min of regional ischemia. The length of completely non-functioning myocardium is theoretically reflected by the value of L_0 (Hooke's law; Atkins, 1970). Thus, our finding that L_0 was increased by regional ischemia theoretically corresponds to an increase of non-functioning myocardium in the ischemic region.

With the present model, the myocardium can be classified as ''functioning'' or ''non-functioning''. On the force–length plane of myocardial contraction, E_c apparently represents the average contractility of the overall functioning (i.e., contracting) myocardium irrespective of its level of contractility, while any myocardium that is non-functional (and thus not contracting) is theoretically not reflected by E_c. Therefore, the contractility of the non-ischemic functioning myocardium apparently increased during regional ischemia because E_c increased, although the model cannot identify the specific part of the left ventricular wall where regional myocardial contractility was enhanced.

The decrease of the left ventricular ejection fraction with regional ischemia reflected the increase of the left ventricular end-systolic and end-diastolic volumes while the stroke volume remained unchanged. The lack of a decrease in cardiac output during regional ischemia may be accounted for by assuming that the left ventricle underwent dilation due to an increase of non-contracting ischemic myocardium and operation of the Frank–Starling mechanism. In addition, the contractility of the functioning non-ischemic myocardium may have increased due to an increase of intracellular Ca^{2+} and/or an increase in the force generated by each active cross-bridge.

The finding that K_a was not decreased despite an increase of the end-diastolic myocardial length after ischemia suggests that the kinetic constant (K_a) of Ca^{2+} binding to troponin C may show a relative increase during ischemia, as does E_c. Since autonomic blockade was used in the present study, any potential effect of cardiac sympathetic nervous activity on the relative increase in E_c and K_a during regional myocardial ischemia should have been eliminated.

4.2. Force–length–time relation after reperfusion

L_0 decreased significantly after reperfusion. It appears that reactivation of the non-functioning myocardium by reperfusion was reflected in this change of L_0. After reperfusion, the left ventricular end-diastolic and end-systolic volumes decreased, while E_c remained at the same level as that during ischemia. These data suggest that the contractility of the recovering myocardium was so low that overall myocardial contractility was not significant altered when the weak post-ischemic region and the non-ischemic hyperfunctioning region were combined.

Neither L_0 nor E_c was restored to the pre-ischemic value after 15 min of reperfusion. This indicates that myocardium subjected to 15 min of ischemia did not show complete functional recovery after 15 min of reperfusion, i.e., it was ''stunned'' myocardium.

4.3. Effect of angiotensin-converting enzyme inhibitor infusion on the force–length–time relation during ischemia–reperfusion

In the imidapril group, L_0 showed a pattern of changes similar to those noted in the control group during ischemia and reperfusion, but the percent increase of L_0 after reperfusion was significantly smaller than in the control group (Fig. 3b). Since L_0 reflects the length of non-functioning myocardium, the percent decrease of L_0 after reperfusion was significantly greater in the imidapril group than in the control group. Much information is already available concerning the protective effect of angiotensin-converting enzyme inhibitors against myocardial ischemia–reperfusion injury (Ertl et al., 1982, 1983; Lefer and Peck, 1984; Hock et al., 1985; De Graeff et al., 1987, 1988; Martorana et al., 1990; Noda et al., 1993; Hartmann et al., 1993; Node et al., 1998), and these findings indicate that imidapril has a similar protective effect.

Interestingly, in contrast to the control group, the imidapril group showed no significant change in E_c during ischemia–reperfusion (Figs. 2 and 3a). Our model suggests

that the increase of E_c in the control group during ischemia may be due to (1) an increase in the intracellular Ca^{2+} concentration, (2) an increase in the force (f) delivered per cross-bridge, or (3) an increase in the affinity α of Ca^{2+} for troponin C in the functioning myocardium (Takeda et al., 1991a). Angiotensin-converting enzyme inhibitors potentiate the endothelium-dependent hyperdepolarization induced by opening of Ca^{2+}-activated K^+ channels through an increase in bradykinin (Cowan and Cohen, 1991; Mombouli et al., 1992; Illiano et al., 1994; Node et al., 1998), which may attenuate the Ca^{2+} overload during ischemia–reperfusion. Thus, E_c presumably did not increase in the imidapril group because of the attenuation of the Ca^{2+} overload in the functioning myocardium.

There was no significant change of K_a with ischemia–reperfusion. According to our model, K_a decreases as the length of the myocardium increases. In addition, K_a increases as the cAMP level increases (Kurihara and Konishi, 1987), but is not dependent on the intracellular Ca^{2+} concentration (Sonnenblick, 1962; Morgan et al., 1983; Blinks and Endoh, 1986). The net effect of these two mechanisms, and possibly of other unknown factors, apparently caused K_a to remain unchanged in both groups.

In earlier studies, captopril reduced the extent of myocardial necrosis after 6 h of coronary artery occlusion in dogs through an increase in regional myocardial blood flow (Ertl et al., 1982, 1983). Enalapril has also been shown to reduce myocardial infarct size in rats subjected to 24 h of complete coronary artery occlusion (Hock et al., 1985). Since L_0 reflects the length of non-functioning ischemic myocardium, these reports lend support to our results. The protective effect of angiotensin-converting enzyme inhibitors during ischemia has been much discussed (Ertl et al., 1982, 1983; Lefer and Peck, 1984; Hock et al., 1985; De Graeff et al., 1987, 1988; Martorana et al., 1990; Hartmann et al., 1993; Noda et al., 1993; Node et al., 1998). The most likely explanation for the protective effect of angiotensin-converting enzyme inhibitors during ischemia is an increase in the production of bradykinin, as suggested by (1) increased bradykinin levels in the vein draining the ischemic area and (2) abolition of the protective effects of angiotensin-converting enzyme inhibitors by the bradykinin B_2 receptor antagonist, icatibant acetate (HOE 140). Bradykinin presumably induces the release of nitric oxide and prostacyclin from vascular endothelial cells, leading to a subsequent increase in cyclic guanosine monophosphate (cGMP), vasodilation, inhibition of platelet adhesion and aggregation, and increased glucose uptake. In the present study, the percent increase of L_0 after reperfusion was smaller in the imidapril group than in the control group and a significant increase in the plasma bradykinin level in the coronary sinus was noted during ischemia–reperfusion. Taken together, these results suggest that the bradykinin level increased by imidapril may play a role in the reduction of myocardial damage during ischemia–reperfusion in dogs.

4.4. Antiarrhythmic effect of angiotensin-converting enzyme inhibitor infusion on ischemic myocardium

In the present study, imidapril reduced the mortality rate that results from reperfusion-induced ventricular arrhythmias. The earliest experimental evidence that angiotensin-converting enzyme inhibitors reduce reperfusion-related ventricular arrhythmias in isolated perfused rat hearts (Van Gilst et al., 1984, 1986; De Graeff et al., 1986; Li and Chen, 1987; Linz and Scholkens, 1987; Rochette et al., 1987; Fleetwood et al., 1991; Arad et al., 1992) is consistent with the present results. The antiarrhythmic effects of angiotensin-converting enzyme inhibitors have also been shown in vivo in the rat (Coker and McGrath, 1985; Rochette et al., 1987), dog (Elfellah and Ogilvie, 1985; Westlin and Mullane, 1988), and pig (Muller et al., 1989) and in coronary artery occlusion models. Angiotensin-converting enzyme inhibitors do not alter the action potential duration, unlike traditional antiarrhythmic drugs (Hemsworth et al., 1989; Linz et al., 1989). The most likely explanation for the antiarrhythmic effect of angiotensin-converting enzyme inhibitors is their ability to prevent the breakdown of bradykinin. In the present study, imidapril also significantly increased the bradykinin concentration of coronary venous blood (Fig. 4a). Linz et al. (1992) reported that reperfusion arrhythmias in isolated rat hearts are reduced by bradykinin and that this protection is abolished by either the inhibitor of the L-arginine nitric oxide pathway, N^G-nitro-L-arginine methyl ester (L-NAME), or by the selective bradykinin B_2 receptor antagonist, icatibant acetate (HOE 140). Thus, the antiarrhythmic effect of angiotensin-converting enzyme inhibitors may be the result of a decrease in the severity of ischemia through the prevention of bradykinin breakdown. However, the exact cellular mechanism of this effect needs further study.

4.5. Limitations of the present study

Intravenous administration of propranolol is usually employed to prevent major reflex changes of autonomic tone over the course of caval occlusion (Little et al., 1985). Although propranolol was administered to the three groups of dogs, it cannot be denied that this procedure may have induced bias in the present study.

4.6. General conclusion

The force–length relationship showed that the contractility of functioning myocardium determined from E_c and the length of non-functioning myocardium determined from L_0 were both increased during regional myocardial ischemia. These findings suggest that the average contractility of average myocardium increased despite the increased non-functioning myocardium. The increase in E_c and L_0 during ischemia–reperfusion in the control group was pre-

vented by the infusion of imidapril, associated with an increase in bradykinin. These findings suggest that imidapril has a protective effect against myocardial ischemia–reperfusion injury not only through the reduction of infarct size, but also through the restoration of increased overall myocardial contractility with the increase in bradykinin.

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