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European Journal of Pharmacology 382 (1999) 187–196

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Participation of angiotensin II and bradykinin in contractile function in dog stunned myocardium

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Received 26 May 1999; received in revised form 5 August 1999; accepted 10 August 1999

Abstract

We examined the effects of enalapril and 4′-[(1,4′-dimethyl-2′-propyl-[2,6′-bi-1H-enzimidazole]-1′-yl)methyl]-[1,1′-biphenyl]-2-carboxylic acid (BIBR-277), an angiotensin II receptor antagonist, on contractile dysfunction in the stunned myocardium. Dogs were subjected to 20-min ligation of the coronary artery, followed by 60-min reperfusion. Saline, enalapril (1 mg/kg or 3 mg/kg), or BIBR-277 (3 mg/kg) was injected i.v. 10 min before ligation. D-Arginyl-L-arginlyl-L-prolyl-*trans*-4-hydroxy-L-prolylglycyl-3-(2-thienyl)-L-alanyl-L-seryl-D-1,2,3,4-tetrahydro-3-isoquinolinecarbonyl-L-(2α, 3β, 7aβ)-octahydro-1H-indole-2-carbonyl-L-arginine (Hoe-140), a bradykinin B_2 receptor antagonist, at 300 μg/kg was injected i.v. 10 min before drug injection. Contractile function was assessed on the basis of percentage segment shortening (%SS). ATP levels were measured in 60-min reperfused hearts. %SS significantly decreased during ischemia, and recovered during reperfusion, although the %SS was significantly less than the pre-ischemic level. Both enalapril at either dose and BIBR-277 significantly enhanced %SS recovery during reperfusion, an effect which was associated with a tendency toward energy preservation. Hoe-140 completely abolished the effect of enalapril at either dose, while it did not modify that of BIBR-277. Inhibition of angiotensin II formation and bradykinin breakdown may be separately related to the improvement of myocardial stunning.

Keywords: Myocardial stunning; Enalapril; BIBR-277; Hoe-140

1. Introduction

Angiotensin converting enzyme inhibitors succeed in the treatment of patients with hypertension and congestive heart failure (Packer, 1987; Robertson and Tillman, 1987). This group of drugs also protects the myocardium from ischemia and reperfusion injury in experimental animals (Ertl, 1988; Przyklenk and Kloner, 1989). Recently, we have reported that 4′-[(1,4′-dimethyl-2′-propyl-[2,6′-bi-1H-enzimidazole]-1′-yl)methyl]-[1,1′-biphenyl]-2-carboxylic acid (BIBR-277), an angiotensin II type 1 receptor-selective antagonist, significantly improves the recovery of myocardial contractile function during reperfusion after brief ischemia in a dose-dependent manner (Izumi et al., 1996). Although angiotensin II may restore the blood pressure that decreases due to failure of cardiac contractile function, and may increase cell proliferation to repair the injured cells (Katoh et al., 1989), it also increases coronary vascular resistance and myocardial necrosis (Gavras et al., 1975). In fact, the renin–angiotensin system is activated following acute myocardial infarction in both humans (Mcalpine and Cobbe, 1988) and experimental animals (Ertl et al., 1982; Liang et al., 1982). Because the angiotensin converting enzyme is identical with kininase II (Erdos and Skidgel, 1987), enalapril decreases the plasma level of angiotensin II and increases that of bradykinin. It is possible that inhibition of both angiotensin II formation and bradykinin breakdown is involved in the mechanisms of myocardial protection against ischemic injury by enalapril.

Since the localized angiotensin II may be formed by the chymase but not the angiotensin I converting enzyme (Okunishi et al., 1987; Kinoshita et al., 1991), some deleterious effects on the ischemic/reperfused myocardium may be produced by the locally produced angiotensin II, even in the presence of enalapril. BIBR-277 potentially

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00586-5

interacts with angiotensin type 1 receptors, but not with type 2 receptors (Wienen et al., 1993). BIBR-277 can antagonize the angiotensin II receptor stimulation due to angiotensin II formed via either angiotensin I converting enzyme or the chymase-related pathway. These findings led us to consider what is the main mechanism involved in the improvement of cardiac contractile dysfunction in the stunned myocardium, increasing bradykinin level or decreasing circulating and locally formed angiotensin II. In the present study, therefore, we studied the effects of enalapril and BIBR-277 on contractile and metabolic dysfunction in stunned myocardium in the presence and absence of D-arginyl-L-arginlyl-L-prolyl-*trans*-4-hydroxy-L-prolylglycyl-3-(2-thienyl)-L-alanyl-L-seryl-D-1,2,3,4-tetrahydro-3-isoquinolinecarbonyl-L-(2α,3β,7aβ)-octahydro-1H-indole-2-carbonyl-L-arginine (Hoe-140), a selective bradykinin B_2 receptor antagonist.

2. Materials and methods

The investigation conformed with the *Guide for the care and use of laboratory animals* [U.S. National Institutes of Health (NIH) publication No. 85-23, revised 1985].

2.1. Preparation of animals

Healthy mongrel dogs of either sex weighing 6–30 kg each were anesthetized with 30 mg/kg sodium pentobarbital i.v., and ventilated with room air. Left thoracotomy was performed between the fourth and fifth ribs, and the ventricle was exposed. After the heart had been suspended in a pericardial cradle, the main trunk of the left anterior descending coronary artery was dissected free from the distal end to the first diagonal branch and was loosely encircled with a silk thread ligature. The left anterior descending coronary flow was measured using a magnetic flow probe positioned in the left anterior descending coronary artery proximal to the ligature. To measure the left ventricular end diastolic pressure and the first derivative of left ventricular pressure, a polyethylene tube connected to a pressure transducer was inserted into the left ventricular chamber through the cardiac apex. A pair of ultrasonic crystals was implanted in a circumferential plane in the left anterior descending coronary artery region. The two crystals of each pair were separated by about 1 cm. The percentage segment shortening (%SS) was calculated according to the equation $\%SS = [(\text{diastolic segment length} - \text{systolic segment length})/\text{diastolic segment length}] \times 100$. The diastolic segment length was determined at the beginning of the rising phase of positive first derivative of left ventricular pressure (the onset of isovolumic contraction) and the systolic segment length was determined at the peak negative first derivative of left ventricular pressure. Arterial blood pressure was measured via a cannula introduced from the left femoral artery and advanced to a point near the aortic arch. The heart rate was counted from the R wave of the ECG limb lead II.

After control observations had been completed, either saline, 1 and 3 mg/kg of enalapril, or 3 mg/kg of BIBR-277 was injected from the left femoral vein over a period of 30 s. Because enalapril, 1 mg/kg, can exert a beneficial effect on ischemic/reperfused hearts (Barbe et al., 1996) and a BIBR-277 dose of 3 mg/kg shows the most potent effect on the stunned myocardium in our previous paper (Izumi et al., 1996), we chose as dosages 1 mg/kg of enalapril and 3 mg/kg of BIBR-277 for the present study. To confirm the inhibition of the converting enzyme, the higher dose of enalapril at 3 mg/kg was also tested. The ligature around the left anterior descending coronary artery was tied 10 min after the injection and then released 20 min after the ligation. Hemodynamic parameters were measured for a further 60 min. In the experiment with a bradykinin B_2 receptor antagonist, 300 μg/kg of Hoe-140, was injected i.v. 10 min before starting saline ($n = 6$), 1 mg/kg of enalapril ($n = 6$), 3 mg/kg of enalapril ($n = 6$), or 3mg/kg of BIBR-277 ($n = 6$) injection. The antagonistic potency of Hoe-140 was confirmed with a bradykinin (10 μg/kg) i.v. injection before and 8 min after Hoe-140 injection. A full-thickness transmural sample of the myocardium was taken from the left anterior descending coronary artery region 60 min after reperfusion. Ischemia and reperfusion lasted 20 and 60 min, respectively. The myocardial samples were immediately pressed and frozen with clamps chilled in liquid nitrogen. The subendocardial portion of the frozen sample was used for biochemical assays (Ichihara and Abiko, 1975).

2.2. Assay of tissue metabolites

The frozen subendocardial samples were pulverized in a mortar with pestle precooled in liquid nitrogen and were extracted with 3 vol 6% perchloric acid. The levels of ATP, ADP, AMP, creatine phosphate and lactate were determined in the neutralized perchloric acid extracts, according to standard enzymatic procedures (Bergmeyer, 1974). Non-ischemic and 20-min ischemic values for the tissue metabolites were determined for an additional 10 dogs.

2.3. Statistical analysis

Values are expressed as means ± S.E.M. The significance of differences in hemodynamics between groups was evaluated by two-way analysis of variance for repeated measurements, and then by Dunnett's *t*-test for the corresponding time points. Differences in hemodynamics within groups were compared using Student's paired *t*-test. The

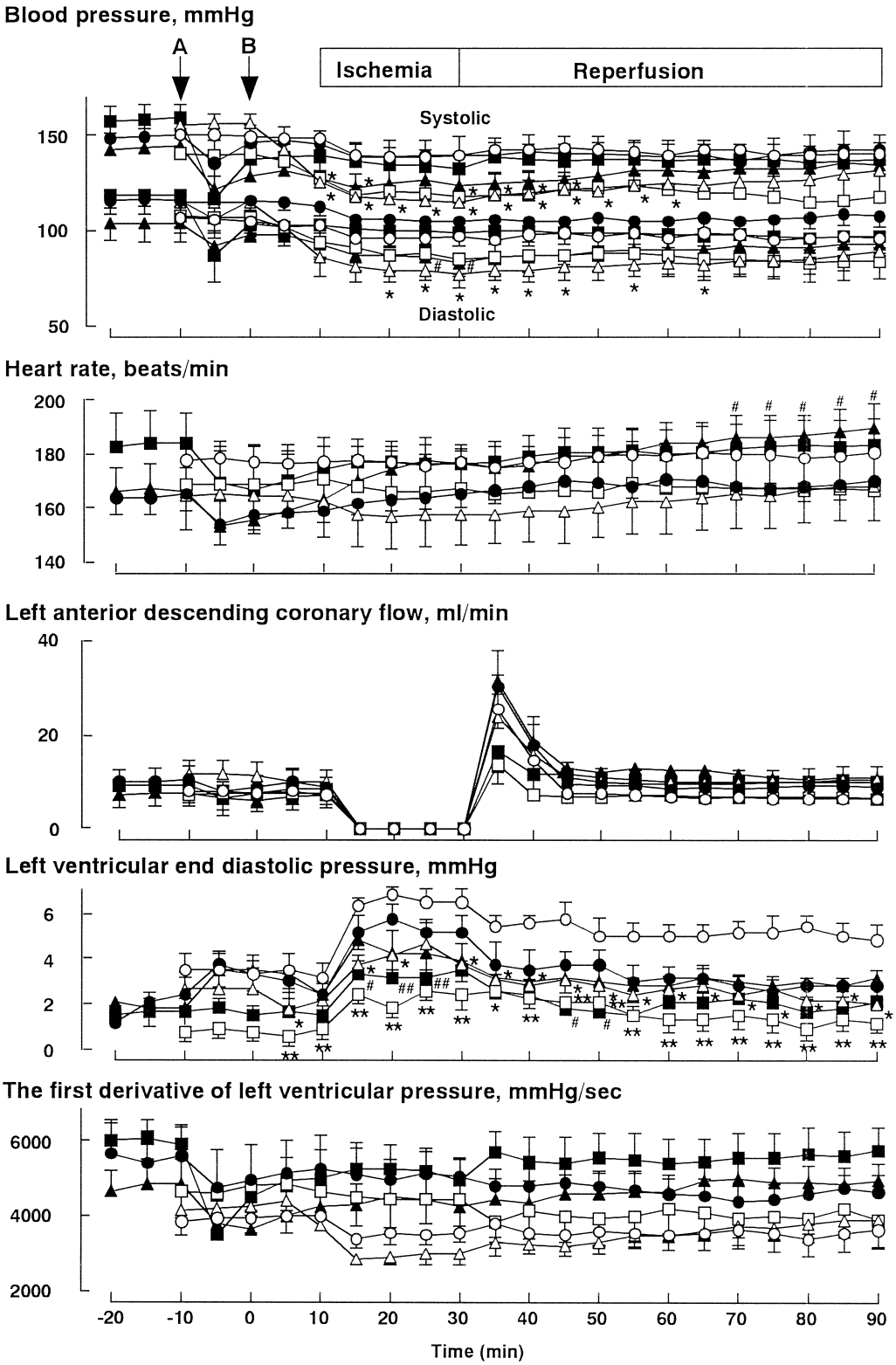


Fig. 1. The effect of enalapril on changes in blood pressures, heart rate, left anterior descending coronary flow, left ventricular end diastolic pressure, and the first derivative of left ventricular pressure during ischemia and reperfusion. After 10 min of control measurement, either saline (○), enalapril at 1 mg/kg (△), or enalapril at 3 mg/kg (□) was injected i.v. (arrow B). Hoe-140 at 300 μg/kg was injected i.v. (arrow A) 10 min before injection of saline (●), enalapril at 1 mg/kg (▲), and enalapril at 3 mg/kg (■). The left anterior descending coronary artery was ligated 10 min after either saline or enalapril injection. After 20 min of left anterior descending coronary artery ligation, the ligated left anterior descending coronary artery was released, so that the ischemic myocardium was reperfused. Symbols indicating significance within a group are not shown to avoid complexity. $^{*}P < 0.05$; $^{**}P < 0.01$, compared with the corresponding values for the saline group. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$, compared with the corresponding values for the saline + Hoe-140 group.

levels of myocardial energy metabolites between groups were compared using an unpaired *t*-test. Differences in mortality between groups were analyzed with a χ^2 test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Mortality and exclusion

Initially, 78 dogs were used, but 30 were excluded because of ventricular fibrillation during ischemia or reperfusion (five dogs in the saline group, five dogs in the enalapril 1 mg/kg group, four dogs in the BIBR-277 group, 10 dogs in the saline + Hoe-140 group, five dogs in the enalapril 1 mg/kg + Hoe-140 group, and one dog in the BIBR-277 + Hoe-140 group). Thus, 48 dogs were used for data analysis ($n = 6$ for the saline group, $n = 5$ for the enalapril 1 mg/kg group, $n = 6$ for the enalapril 3 mg/kg group, $n = 7$ for the BIBR-277 group, $n = 6$ for the saline + Hoe-140 group, $n = 6$ for the enalapril 1 mg/kg + Hoe-140 group, $n = 6$ for the enalapril 3 mg/kg + Hoe-140 group, and $n = 6$ for the BIBR-277 + Hoe-140 group). Because no dogs died in the enalapril 3-mg/kg groups either with or without Hoe-140, enalapril at 3 mg/kg significantly reduced mortality as compared to that in the corresponding saline group. There were no significant differences in mortality between the saline and enalapril 1-mg/kg or BIBR-277 groups.

3.2. Hemodynamic changes

Changes in systolic and diastolic blood pressures, heart rate, left anterior descending coronary flow, left ventricular end diastolic pressure, and the first derivative of left ventricular pressure during ischemia and reperfusion in the enalapril and enalapril + Hoe-140 groups are illustrated in Fig. 1. Enalapril was tested at dosages of 1 and 3 mg/kg. In the saline group, systolic and diastolic blood pressures slightly but significantly decreased during ischemia, and returned to their pre-ischemic levels during reperfusion. Heart rate did not change throughout the experiment. Left anterior descending coronary flow decreased to 0 ml/min during ischemia and showed a transient and overshoot increase just after reperfusion. The left anterior descending coronary flow 5 and 10 min after reperfusion was significantly higher than the pre-ischemic flow. Left ventricular end diastolic pressure was significantly increased during ischemia and the increased left ventricular end diastolic pressure was sustained during reperfusion. The first derivative of left ventricular pressure significantly decreased during ischemia and gradually returned toward its pre-ischemic level during reperfusion. Injection of enalapril at either 1 mg/kg or 3 mg/kg significantly decreased the systolic and diastolic blood pressures. Systolic and diastolic pressures in the enalapril 1-mg/kg group and systolic pressure in the enalapril 3-mg/kg group were significantly lower than those in the saline group. The increased left ventricular end diastolic pressure during ischemia and reperfusion in the enalapril groups was significantly lower than that in the saline group. Changes in heart rate, left anterior descending coronary flow, and the first derivative of left ventricular pressure due to ischemia and reperfusion were not modified by enalapril at either dose.

Injection of Hoe-140 transiently decreased systolic and diastolic blood pressures, significantly decreased heart rate and the first derivative of left ventricular pressure, and significantly increased left ventricular end diastolic pressure. Enalapril injection at either dose did not decrease systolic and diastolic blood pressures when Hoe-140 was given as pretreatment. Even in the presence of Hoe-140, enalapril attenuated the increase in left ventricular end diastolic pressure due to ischemia and reperfusion. Hoe-140 did not affect heart rate, left anterior descending coronary flow and the first derivative of left ventricular pressure in the enalapril 1-mg/kg and 3-mg/kg groups.

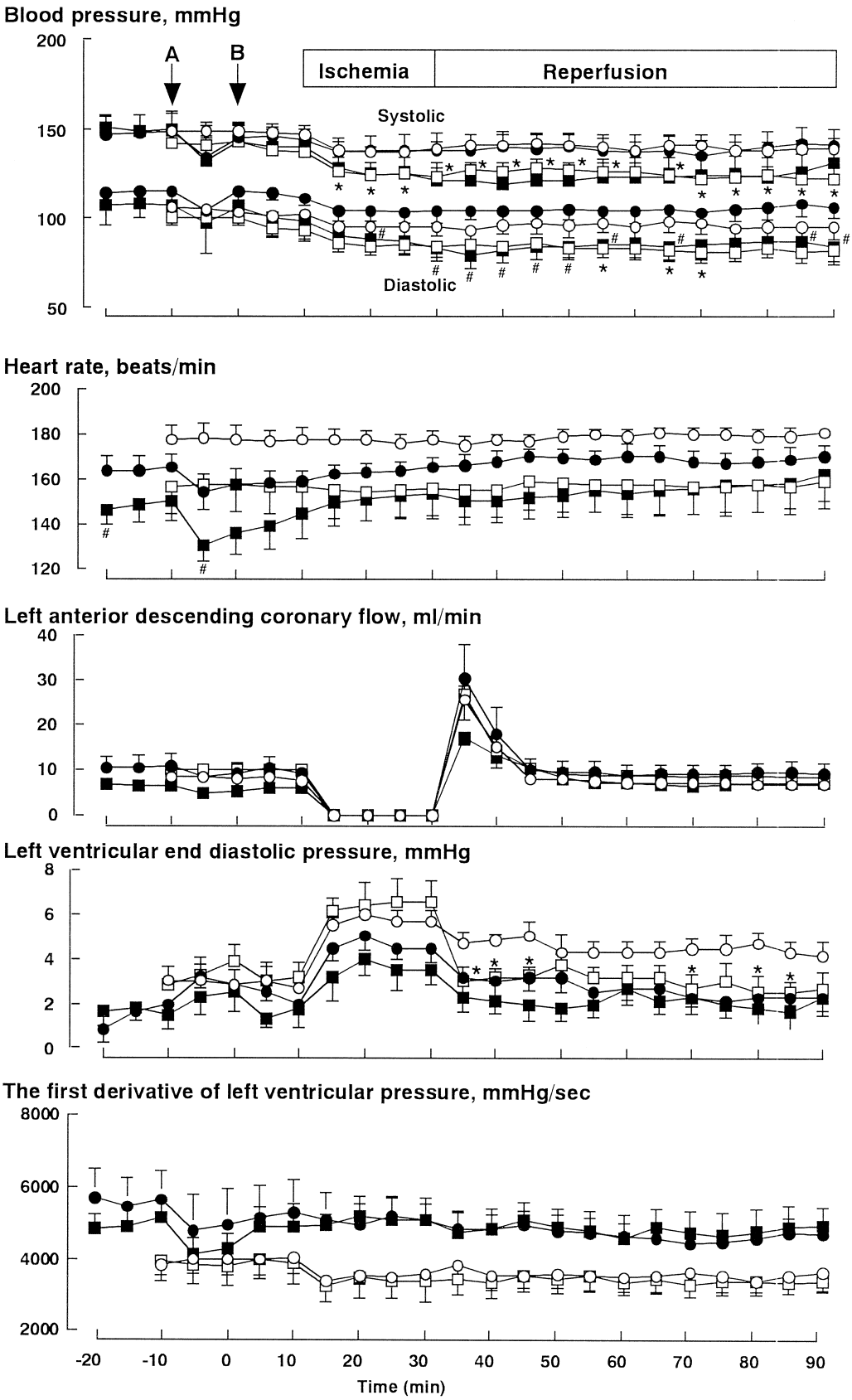
The hemodynamics of the BIBR-277 and BIBR-277 + Hoe-140 groups are illustrated in Fig. 2. Values in the saline and saline + Hoe-140 groups are cited from those in Fig. 1. In the BIBR-277 group, systolic and diastolic blood pressures decreased significantly 10 min after BIBR-277 injection, and decreased further during ischemia. The decreases in blood pressures were sustained even after reperfusion. Changes in heart rate, left anterior descending coronary flow, left ventricular end diastolic pressure and the first derivative of left ventricular pressure during ischemia and reperfusion were not modified by BIBR-277. Hemodynamic changes caused by BIBR-277 injection, ischemia or reperfusion were not altered by the pretreatment with Hoe-140.

3.3. Changes in %SS

Changes in %SS during ischemia and reperfusion in the enalapril and enalapril + Hoe-140 groups are shown in Fig. 3. The values for %SS were normalized to their pre-injection values with either enalapril or Hoe-140. In the saline group, %SS decreased significantly and fell

Fig. 2. The effect of BIBR-277 on changes in blood pressures, heart rate, left anterior descending coronary flow, left ventricular end diastolic pressure, and the first derivative of left ventricular pressure during ischemia and reperfusion. After 10 min of control measurement, either saline (○), or BIBR-277, 3 mg/kg (□) was injected i.v. (arrow B). Hoe-140 was injected i.v. (arrow B) 10 min before saline (●) or BIBR-277 (■) injection. Values in the saline and saline + Hoe-140 groups are cited from those in Fig. 1. Experimental protocol was the same as that for Fig. 1. Symbols indicating significance within a group are not shown to avoid complexity. $^{*}P < 0.05$, compared with the corresponding values for the saline group. $^{\#}P < 0.05$, compared with the corresponding values for the saline + Hoe-140 group.

below 0% during ischemia, indicating bulging. Reperfusion restored the %SS that had been decreased by ischemia toward its pre-ischemic level, but the recovery was incomplete. Although enalapril (1 mg/kg or 3 mg/kg) did not



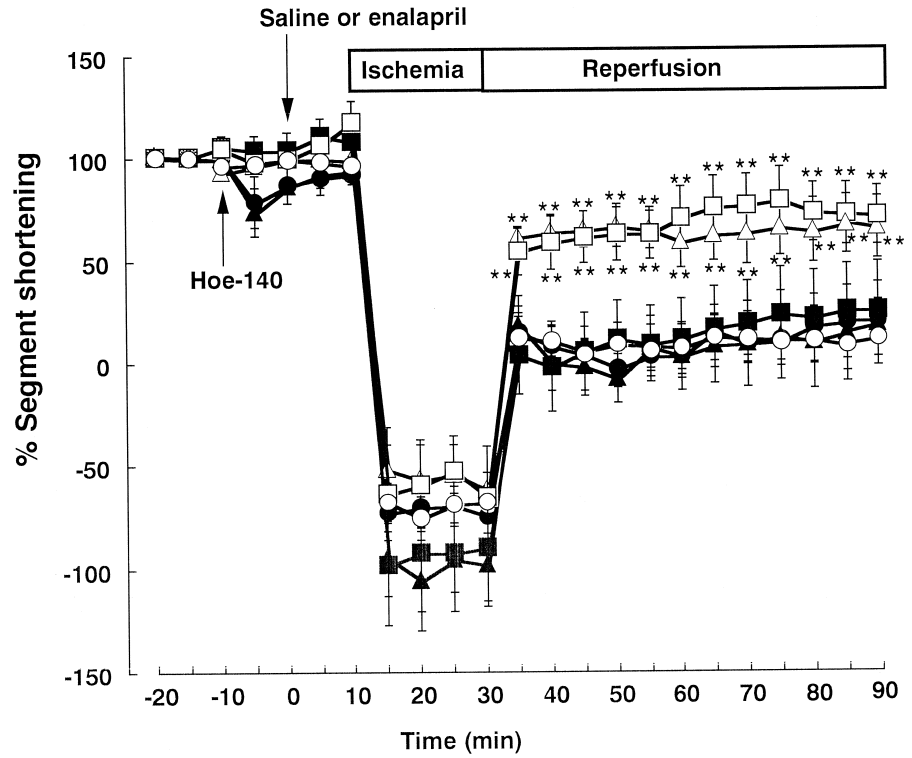


Fig. 3. The effect of enalapril on changes in %SS during ischemia and reperfusion with or without Hoe-140. Changes in %SS are expressed as percentage of the value obtained just before saline or enalapril injection, or Hoe-140 injection. Symbols and experimental protocol are the same as those in Fig. 1. Symbols indicating significance within a group, and between with and without Hoe-140 groups are not shown to avoid complexity. $^{**}P < 0.01$, compared with the corresponding values for the saline group.

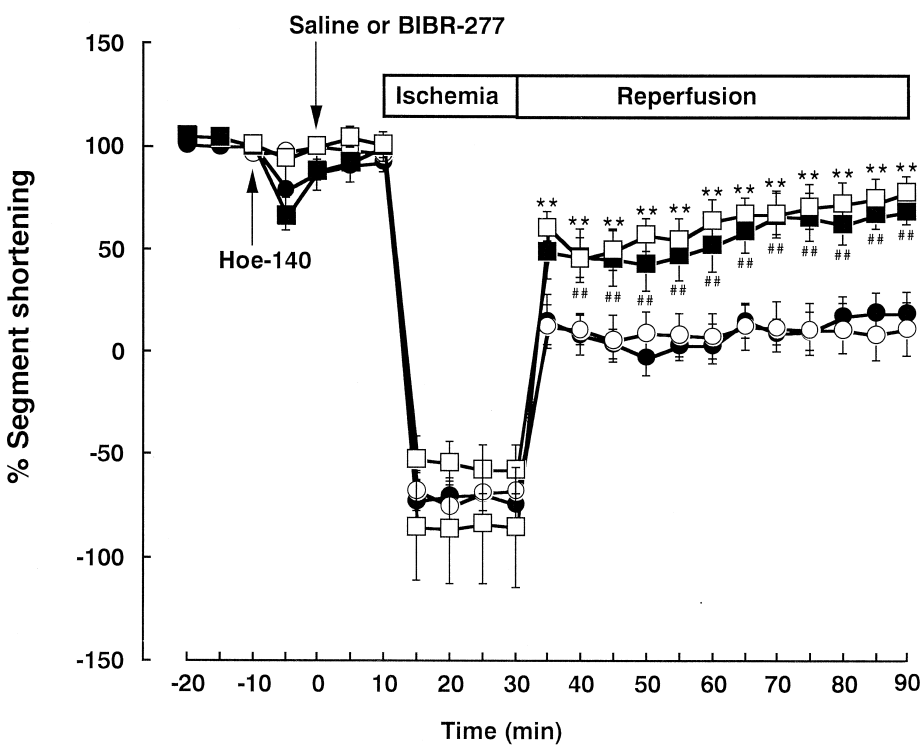


Fig. 4. The effect of BIBR-277 on changes in the %SS during ischemia and reperfusion with or without Hoe-140. Values for the saline and saline + Hoe-140 groups are cited from those in Fig. 3. Symbols and experimental protocol are the same as those in Fig. 2. Symbols indicating significance within a group are not shown to avoid complexity. $^{**}P < 0.01$, compared with the corresponding values for the saline group. $^{\#\#}P < 0.01$, compared with the corresponding values for the saline + Hoe-140 group.

Table 1
Effects of Hoe-140 on bradykinin-induced hemodynamic changes
In the experiment with Hoe-140, bradykinin, 10 μg/kg, was injected i.v. twice before and 8 min after Hoe-140, 300 μg/kg, injection. Data are means ± S.E.M. of 23 observations (one set of data out of 24 is missing).

	Before bradykinin	After bradykinin	Pretreatment with Hoe-140	
			Before bradykinin	After bradykinin
Systolic blood pressure (mm Hg)	151 ± 5	105 ± 4*	134 ± 6	136 ± 5
Diastolic blood pressure (mm Hg)	109 ± 4	54 ± 3*	100 ± 5	103 ± 4**
Heart rate (beats/min)	167 ± 5	186 ± 8*	153 ± 5	153 ± 5
Left anterior descending coronary flow (ml/min)	8.2 ± 1.2	13.4 ± 1.7*	6.0 ± 1.1	6.4 ± 1.0**

*$P < 0.01$, vs. before bradykinin injection.
**$P < 0.05$.

modify the %SS measured before and during ischemia, it significantly enhanced the recovery of %SS during reperfusion. A transient but significant decrease in %SS was observed just after Hoe-140 injection. Hoe-140 itself did not alter the changes in %SS during ischemia and reperfusion. Pretreatment with Hoe-140 almost completely abolished the enhancement of %SS recovery by enalapril during reperfusion.

Fig. 4 shows the effects of BIBR-277 and BIBR-277 with Hoe-140 on %SS during ischemia and reperfusion. BIBR-277 also improved contractile dysfunction during reperfusion after ischemia. Pretreatment with Hoe-140 did not modify the effect of BIBR-277 on changes in %SS during ischemia and reperfusion.

3.4. Hemodynamic changes due to bradykinin injection

To confirm the antagonism of Hoe-140 to bradykinin B_2 receptors, bradykinin, 10 μg/kg, was injected i.v. before and after Hoe-140 treatment (Table 1). Bradykinin significantly decreased systolic and diastolic blood pressures, and significantly increased heart rate and left anterior descending coronary flow. Complete inhibition of the bradykinin-induced response was observed 8 min after Hoe-140 injection.

Table 2
Effects of enalapril and BIBR-277 on myocardial energy metabolites
The heart was made ischemic for 20 min by ligating the left anterior descending coronary artery in anesthetized open-chest dogs followed by 60 min of reperfusion. Either saline, enalapril (1 mg/kg and 3 mg/kg), or BIBR-277 (3 mg/kg) was injected i.v. 10 min before the onset of ischemia. Hoe-140, when used, was injected i.v. 10 min before saline, enalapril, or BIBR-277 injection, i.e., 20 min before the onset of ischemia. The subendocardial tissue samples were taken from a previously ischemic region 60 min after reperfusion. Data are means ± S.E.M. n = Number of observations.

Treatment	n	ATP	ADP	AMP	Creatine phosphate	Lactate
		(μmol/g wet weight)				
Non-ischemic hearts						
Saline	5	5.04 ± 0.09	0.96 ± 0.05	0.14 ± 0.01	5.83 ± 1.11	2.30 ± 0.49
20-min ischemic hearts						
Saline	5	2.28 ± 0.27*	0.81 ± 0.04**	0.13 ± 0.01	2.13 ± 0.51*	18.55 ± 2.36*
60-min reperfused hearts						
Saline	6	3.03 ± 0.22*	0.67 ± 0.04*	0.10 ± 0.01*	9.41 ± 0.61*	1.94 ± 0.30
Saline + Hoe-140	6	3.23 ± 0.19	0.64 ± 0.02	0.13 ± 0.01****	11.33 ± 0.49****	0.45 ± 0.06***
Enalapril (1 mg/kg)	5	3.60 ± 0.21	0.70 ± 0.04	0.08 ± 0.01	8.49 ± 0.27	2.26 ± 0.43
Enalapril (1 mg/kg) + Hoe-140	6	3.06 ± 0.25	0.60 ± 0.04	0.16 ± 0.01******	11.42 ± 0.85	1.03 ± 0.22******
Enalapril (3 mg/kg)	6	3.80 ± 0.24****	0.71 ± 0.05	0.15 ± 0.01***	8.31 ± 0.94	1.21 ± 0.31
Enalapril (3 mg/kg) + Hoe-140	6	3.18 ± 0.28	0.63 ± 0.04	0.17 ± 0.02******	9.57 ± 0.88	0.71 ± 0.11
BIBR-277	7	3.47 ± 0.38	0.71 ± 0.06	0.11 ± 0.01	8.40 ± 0.71	1.94 ± 0.21
BIBR-277 + Hoe-140	6	3.55 ± 0.27	0.73 ± 0.06	0.21 ± 0.01*****	9.15 ± 0.94	0.92 ± 0.08*****

*$P < 0.01$, vs. non-ischemic saline-treated group.
**$P < 0.05$
***$P < 0.01$, vs. 60-min reperfused saline-treated group.
****$P < 0.05$
*****$P < 0.01$, vs. saline + Hoe-140-treated group.
******$P < 0.05$

3.5. Metabolic changes

The levels of energy metabolites in the 60-min reperfused heart are summarized in Table 2. The values for non-ischemic hearts and 20-min ischemic hearts obtained from additional untreated sham-operated and 20-min ischemic hearts, respectively, are also shown. Ischemia for 20 min significantly decreased the levels of ATP and creatine phosphate, and increased the level of lactate. Reperfusion for 60 min returned the level of lactate to its pre-ischemic value. However, although the level of ATP tended to increase after 60 min of reperfusion, it was still significantly lower than that in the non-ischemic heart. The overshoot recovery of the creatine phosphate level was observed 60 min after reperfusion. Neither enalapril, 1 mg/kg, nor BIBR-277 significantly affected the levels of energy metabolites in the 60-min reperfused heart. The ATP and AMP levels in the enalapril 3-mg/kg group were significantly higher than those in the saline-treated 60-min reperfused group. Hoe-140 significantly increased the AMP and creatine phosphate levels, and decreased the lactate level as compared with the corresponding level in the absence of Hoe-140. In the presence of Hoe-140, either enalapril at 1 mg/kg or BIBR significantly increased the AMP and lactate level, and enalapril at 3 mg/kg significantly increased the AMP level.

4. Discussion

It is well demonstrated that angiotensin converting enzyme inhibitors and selective angiotensin II type 1 receptor antagonists have beneficial effects on the ischemic/reperfused myocardium (Yoshiyama et al., 1994). We (Izumi et al., 1996) also have reported a dose-dependent improvement of myocardial contraction in the stunned myocardium with BIBR-277, a selective angiotensin II type 1 receptor antagonist. The present study confirmed that both enalapril and BIBR-277 improve myocardial contractile dysfunction during reperfusion after brief ischemia (Figs. 3 and 4). Hoe-140 almost completely blocked the beneficial effects of enalapril, but not that of BIBR-277, on the stunned myocardium. Hoe-140 is a highly potent and selective bradykinin B_2 receptor antagonist (Hock et al., 1991; Wirth et al., 1991). Stimulation of bradykinin B_2 receptors causes a sharp fall in systolic and diastolic blood pressures due to systemic vasodilation that is mediated by endothelial cell-produced nitric oxide (Pelc et al., 1991) and/or release of prostacyclin (Hock et al., 1991). In conscious dogs, intravenous injection of Hoe-140 in doses of 0.01 to 0.1 mg/kg does not affect systolic and diastolic blood pressures, whereas the antagonist at 1 mg/kg decreases blood pressure because of its residual agonistic activity (Wirth et al., 1991). A transient decrease in blood pressure caused by Hoe-140 injection at 0.3 mg/kg was also observed in the present study (Figs. 1 and 2).

Because the angiotensin converting enzyme is identical to kininase II (Erdos and Skidgel, 1987), angiotensin converting enzyme inhibitors can increase the plasma level of bradykinin. Exogenous bradykinin improves cardiac function following ischemia in isolated rat hearts (Schölkens et al., 1987), protects dog heart against ischemia-induced arrhythmias (Vegh et al., 1991), and also limits infarct size in rabbits (Goto et al., 1995). Inhibition of bradykinin breakdown with angiotensin converting enzyme inhibitors also shows cardioprotective effects on the ischemic myocardium (Kitakaze et al., 1995). Bradykinin itself, or the inhibition of bradykinin breakdown may play an important role in protecting the myocardium against ischemic injury.

There are two pathways in the angiotensin II generating system, angiotensin converting enzyme dependent and independent. It has been suggested that chymase is involved in the generation of angiotensin II in the heart in some species, although for the human heart, angiotensin converting enzyme is reported to be by far the most important enzyme of angiotensin II formation (Blais et al., 1997). Gondo et al. (1989) and Noda et al. (1993) have demonstrated that the formation of local angiotensin II during ischemia occurs via the converting enzyme-independent pathway (the chymase-dependent pathway).

We propose a hypothesis to explain the present results. It is based on the assumption that locally formed angiotensin II, but not circulating angiotensin II, has a deleterious effect on myocardial stunning. In the case of enalapril treatment, the increased bradykinin improves %SS recovery, and the improvement is abolished by Hoe-140. When BIBR-277 is administered, improvement of %SS recovery is due to removal of the deleterious effect of local angiotensin II, and the improvement cannot be abolished by Hoe-140. If circulating angiotensin II, which is formed by the converting enzyme, has some deleterious effect on the stunned myocardium, beneficial effects of enalapril should have partly persisted even in the presence of Hoe-140. It was conceivable that a synergistic effect on the stunned myocardium could be observed when both enalapril and BIBR-277 were used simultaneously. However, we did not do these experiments, because it was unlikely that %SS could recover beyond 100% of its pre-ischemic level during reperfusion.

Why did enalapril and BIBR-277 enhance the recovery of %SS during reperfusion after ischemia? Both enalapril and BIBR-277 significantly (paired *t*-test) decreased systolic and diastolic blood pressures without reflex tachycardia. These hypotensive effects may result in reduction of the myocardial oxygen demand, and are responsible for the improvement of %SS recovery during reperfusion. In fact, when the hypotensive effect of enalapril was diminished by Hoe-140, its beneficial effect on %SS recovery was also abolished. On the other hand, the enhancement of cardiac contraction in the stunned myocardium with BIBR-277 was observed when its hypotensive effect still remained, even after Hoe-140 injection. However, although

the reduction of blood pressure in the enalapril group (20–25%) was more marked than that in the BIBR-277 group (5–7%), the magnitude of each improvement of %SS recovery was similar.

Angiotensin II increases myocardial cytosolic Ca^{2+} through activation of phosphatidylinositol turnover (Brown, 1989). Because the increase in cytosolic Ca^{2+} aggravates the ischemic myocardial damage, enalapril and BIBR-277 exert their protective effects on the stunned myocardium through prevention of cytosolic Ca^{2+} accumulation in the myocardial cell. Bradykinin activates nitric oxide–cyclic guanosine monophosphate system (Pelc et al., 1991).

The beneficial effect of enalapril may be attributable to increases in nitric oxide release via accumulation of bradykinin. However, Goto et al. (1995) have demonstrated that the beneficial effect of bradykinin on myocardial infarction is not due to nitric oxide synthesis but to protein kinase activation.

In spite of a significant enhancement of %SS recovery during reperfusion, there was no significant difference in high-energy phosphate levels in 60-min reperfused hearts between saline- and drug-treated groups (Table 1). We cannot yet explain the reason why Hoe-140 increased the levels of AMP and creatine phosphate, and decreased that of lactate.

In conclusion, enalapril and BIBR-277 improve myocardial dysfunction in the stunned myocardium through different mechanisms. We speculate that enalapril exerts its effect via inhibition of bradykinin breakdown, whereas BIBR-277 blocks the deleterious effects of locally produced angiotensin II.

Acknowledgements

BIBR-277 was kindly supplied by Nippon Boehringer Ingelheim.

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