Br. J. Pharmacol. (1993), **110**, 969–974

Comparison of the effects of EXP3174, an angiotensin II antagonist and enalaprilat on myocardial infarct size in anaesthetized dogs

[1]Vincent Richard, Bijan Ghaleh, Alain Berdeaux & [2]Jean-François Giudicelli

Département de Pharmacologie, Faculté de Médecine Paris-Sud, 63 rue Gabriel Péri, 94276 – Le Kremlin Bicêtre Cédex, France

1 In order to determine whether the renin-angiotensin system is involved in myocardial ischaemia-reperfusion injury, we investigated and compared the effects on infarct size of two different drugs which interfere with this system, i.e., an angiotensin II (AT_1) antagonist, EXP3174, and an angiotensin I-converting enzyme inhibitor (ACEI), enalaprilat in a canine model of ischaemia-reperfusion.

2 EXP3174 (0.1 mg kg^{-1}, i.v. followed by 0.02 mg kg^{-1} h^{-1} for 5.5 h) and enalaprilat (0.3 mg kg^{-1}, i.v. followed by 0.06 mg kg^{-1} h^{-1} for 5.5 h) were used in doses inducing a similar level of inhibition (87 ± 4 and 91 ± 3%, respectively) of the pressor responses to angiotensin I. Control animals received saline.

3 Infarct size and area at risk were quantified by *ex vivo* dual coronary perfusion with triphenyltetrazolium chloride and monastral blue dye. Regional myocardial blood flows (ischaemic and nonischaemic, endocardial, epicardial) were assessed by the radioactive microsphere technique.

4 Both EXP3174 and enalaprilat induced a decrease in mean arterial blood pressure. However, non significant changes in regional myocardial blood flows, whether ischaemic or nonischaemic, were observed after administration of either the ACEI or the AT_1 antagonist.

5 The size of the area at risk was similar in the three groups. By direct comparison, there were no significant differences between infarct sizes in the three groups. Furthermore, there was a close inverse relationship between infarct size and transmural mean collateral blood flow in controls, and none of the treatments altered this correlation. Thus, neither EXP3174 nor enalaprilat limited infarct size.

6 These results indicate that activation of the renin-angiotensin system does not contribute to myocyte death in this canine ischaemia/reperfusion model.

Keywords: Angiotensin II; renin-angiotensin system; coronary circulation; infarct size; enalaprilat; EXP3174; AT_1 antagonist; regional myocardial blood flow

Introduction

Among the numerous factors contributing to ischaemia-induced myocardial damage during coronary artery occlusion, the increase in sympathetic tone and the release of catecholamines are known to play a major role (Schömig *et al.*, 1984; Heusch, 1990). However, ischaemia-induced activation of the renin-angiotensin system may also contribute (Ertl, 1987; Ambrosioni & Borghi, 1989). Indeed, angiotensin II has potent coronary vasoconstrictor properties (Heyndrickx *et al.*, 1976) as well as mild positive inotropic effects (Kobayashi *et al.*, 1978) which may jeopardize the ischaemic myocardium by reducing the oxygen supply while increasing the myocardial oxygen demand.

It has been shown that blockade of the renin-angiotensin system by captopril (Ertl *et al.*, 1982), enalapril (Lefer & Peck, 1984; Hock *et al.*, 1985), and ramipril (Martorana *et al.*, 1990) produces a beneficial effect in myocardial ischaemia. However, this cardioprotective effect of angiotensin I-converting enzyme inhibitors (ACEIs) is not universally recognized, as Liang *et al.* (1982) and Daniell *et al.* (1984) have failed to demonstrate any myocardial protection with ACEIs. But, it must be stressed that the pharmacological approach using ACEIs to investigate the effects of the renin-angiotensin system on myocardial ischaemia is indirect and non specific. Indeed, it is not known whether the results obtained are the direct consequence of the blockade of angiotensin II production, or rather that of some of the indirect effects of ACEIs. For example, ACEIs might enhance the cardioprotective effects of endogenous bradykinin (Linder & Heusch, 1990; Martorana *et al.*, 1990; Baumgarten *et al.*, 1993). In addition, sulphydryl containing ACEIs such as captopril may exert cardioprotective effects during ischaemia and reperfusion through their capacity to scavenge oxygen-derived free-radicals (Westlin & Mullane, 1988; Przyklenk & Kloner, 1989; Grover *et al.*, 1991). Thus, the role of the renin-angiotensin system in the myocardial damage induced by ischaemia and reperfusion cannot be evaluated on the sole basis of the results obtained with ACEIs. In this regard, the recent development of non-peptide angiotensin II receptor antagonists should provide a more direct tool for the investigation of the role of this system in myocardial ischaemia/reperfusion injury.

Losartan (DuP 753) is a recently developed nonpeptide angiotensin II (AT_1) antagonist which, unlike peptide antagonists such as saralasin, does not appear to possess partial agonist properties (Chiu *et al.*, 1991). In the rat, losartan generates an active metabolite, EXP3174, which is most likely to be responsible for part of the pharmacological effects of the drug (Wong *et al.*, 1990). In contrast, the haemodynamic effects of losartan in the dog are limited, probably as a result of the weak biotransformation of losartan into EXP3174 in this species (Wong *et al.*, 1991).

The aim of the present study was to evaluate more directly the role of the renin-angiotensin system in ischaemia-reperfusion injury in dogs. For this purpose, we investigated the effects of EXP3174, the active metabolite of losartan, on infarct size in a model of ischaemia-reperfusion. Furthermore, these effects were compared to those of an ACEI, enalaprilat, in order to determine whether the nonspecific properties of ACEIs do or do not add to the potential beneficial effects of renin-angiotensin system blockade on

[1] Present address: Département de Pharmacologie, UFR de Médecine et de Pharmacie de Rouen, Avenue de l'Université, BP 97, 76803, St Etienne due Rouvray, France.
[2] Author for correspondence.

infarct size. Since the renin-angiotensin system plays only a minimal role in the control of vascular tone in normotensive dogs, this study was carried out in dogs pretreated with frusemide in order to activate their renin-angiotensin systems.

Methods

The animal instrumentation and the ensuing experiments were performed in accordance with the official regulations of the French Ministry of Agriculture. Furthermore, in order to assess regional myocardial blood flow and infarct size, we followed the recommendations of the NHLBI cooperative study (Reimer *et al.*, 1985).

Experimental preparation

Experiments were performed on mongrel dogs of either sex, weighing 15–25 kg and in which the renin-angiotensin system was activated by prior treatment with frusemide (two separate i.v. bolus doses of 20 mg kg^{-1} per day for two consecutive days before the experiment). Animals were anaesthetized with 35 mg kg^{-1} i.v. sodium pentobarbitone and ventilated by a Harvard respirator with room air supplemented with low-flow oxygen. Arterial blood gases were maintained within physiological range by adjusting ventilatory variables as needed. A saline-filled catheter was inserted into the right femoral artery and advanced in the ascending thoracic aorta for measurement of arterial blood pressure and for withdrawal of reference arterial blood samples required for determining tissue flow. Another catheter was inserted in the right femoral vein for injection of drugs. A left thoracotomy was performed in the fourth intercostal space and the heart was suspended in a pericardial cradle. A saline-filled catheter was placed into the left atrial appendage for subsequent injections of radioactive microspheres. The left circumflex coronary artery was isolated distal to the atrial appendage but proximal to its first marginal branch and was encircled with a silk suture so that the artery could be occluded with an atraumatic Schwartz vascular clamp. Any dog that developed ventricular fibrillation during either occlusion or reperfusion was cardioverted, if possible, using a Lifepack 8 defibrillator (Physiocontrol, Redmond, WA, U.S.A.). Arterial blood pressure and lead II of the electrocardiogram were continuously monitored on a Gould Recorder 2200 S (Gould Instruments Inc., Cleveland, OH, U.S.A.). Heart rate was calculated from the blood pressure recordings. The animals were allowed to rest at least 20 min before the first microsphere injection.

Measurement of regional myocardial blood flows

The distribution of regional myocardial tissue flow was assessed by the radioactive microspheres technique. At the times indicated in the experimental protocol, approximately 2×10^6 microspheres (diameter 15 ± 3 μm), labelled with ^{46}Sc, ^{103}Ru or ^{141}Ce (New England Nuclear, Boston, Massachusetts, U.S.A.), were injected through the catheter into the left atrial appendage, followed by a 10 ml saline flush. Beginning just before and continuing for 60 s after injection, a reference sample of arterial blood was withdrawn from the femoral artery at a rate of 9 ml min^{-1}. Reference blood samples were collected in 6 separate aliquots in order to ensure that all radioactivity had been cleared from the circulation at the end of the sampling.

Experimental protocol

Infarcts were produced by a 90 min occlusion of the left circumflex coronary artery followed by a 4 h reperfusion. Regional myocardial blood flow was measured before drug administration, 10 min into occlusion and 60 min into reperfusion. At the completion of the surgical preparation, animals were randomly assigned to one of the three experimental groups (saline, enalaprilat or EXP3174). Ten minutes before the end of reperfusion, the pressor response to i.v. administration of angiotensin 1 (300 ng) (Sigma Chimie, la Verpillère, France) was assessed, then the heart was excised.

Postmorten studies

Area at risk and infarct size The anatomical boundaries of the previously ischaemic and nonischaemic vascular beds were defined by dual perfusion of dyes at physiological pressure (120 mmHg) in the left main coronary artery and in the previously occluded circumflex artery (Reimer *et al.*, 1985). The perfusion fluid was sodium phosphate buffer (pH = 7.4) plus 7% dextran (87.000 mol. wt., Sigma Chimie) to which either 1% triphenyltetrazolium chloride (TTC, Sigma Chimie) (for the previously occluded circumflex artery), or 3% monastral blue dye (Sigma Chimie) (left main artery) was added. The heart was then fixed by immersion for 3 days in a large volume of phosphate-buffered formalin. The fixed left ventricle was then sectioned into eight transverse slices which were weighed. Five slices were used for the measurements of area at risk (previously occluded vascular bed) and of infarct size. The boundaries of the area at risk and infarct (TTC negative area) were then traced; the tracings were digitized using a HP scanner (Hewlett-Packard, Les Ulis, France) interfaced to a Macintosh computer, and the area at risk and infarct were quantitated with image analysis software.

Regional blood flows Flow measurements were made in the three remaining slices. These slices were divided into nonischaemic and central ischaemic zones. Lateral and septal border zones were eliminated to avoid measurements from samples containing a mixture of nonischaemic and ischaemic tissue. Each sample was further subdivided into subendocardial, midmyocardial and subepicardial thirds. Regional myocardial blood flows were calculated according to the formula:

$$\dot{Q}\,m = (\dot{Q}\,r \times Cm)/Cr$$

where $\dot{Q}$ m = myocardial blood flow (ml min^{-1}); $\dot{Q}$ r = reference blood flow (ml min^{-1}); Cm = counts min^{-1} in tissue sample; Cr = counts min^{-1} in reference blood sample. Myocardial blood flows were expressed relative to the tissue sample weight (ml min^{-1} g^{-1}).

Drugs

EXP3174 and enalaprilat were a gift of Merck Sharp and Dohme Research Laboratories, West Point, PA, U.S.A. Enalaprilat was dissolved in sterile saline and EXP3174 was dissolved in a mixture of 5% $NaHCO_3$ and 5% dextrose. EXP3174 (0.1 mg kg^{-1}) and enalaprilat (0.3 mg kg^{-1}) were administered as an i.v. bolus immediately after the first microsphere injection (i.e. 30 min before ischaemia), followed by a continuous i.v. infusion (0.02 and 0.06 mg kg^{-1} h^{-1} for EXP 3174 and enalaprilat, respectively) which lasted until the end of the experiment. The doses of EXP3174 and enalaprilat used in the present study were chosen on the basis of our prior experiments in anaesthetized dogs, as those inducing a similar decrease in mean arterial pressure and a similar inhibition of the pressor response to angiotensin I (Richard *et al.*, 1993).

Statistical analysis of data

All values are expressed as mean ± s.e.mean. Comparisons of regional myocardial blood flows, heart rate and mean arterial pressure values were made by a one-way analysis of variance for repeated measures, followed by a Student-Newman-Keuls test for multiple pairwise comparisons. Comparisons of area at risk and infarct size (expressed as percentage of the area at risk as well as percentage of the left ventricle) were made by a one-way analysis of variance. Furthermore, in order to control for the variability in infarct size due to collateral

flow, infarct size was compared among groups by an analysis of covariance with size as a dependent variable and collateral flow as a covariate. A P value <0.05 was considered statistically significant.

Results

Thirty-two dogs initially entered this study. Eight dogs had to be excluded ($n = 1$, 2 and 5 in the control, enalaprilat and EXP3174 groups, respectively) because they developed intractable ventricular fibrillation. Consequently, results are presented for 24 dogs ($n = 8$ in each group).

Effect of intravenous angiotensin I on mean arterial pressure

Intravenous injection of angiotensin I (300 ng) increased mean arterial pressure by 17 ± 3% in control dogs. This pressor response was reduced by 87 ± 4% and 91 ± 3% by EXP3174 and enalaprilat, respectively.

Haemodynamic data

Table 1 shows heart rate values measured in the three groups throughout this study. Baseline heart rate values which were similar in the three groups, were not affected by any of the three treatments.

Table 1 also shows mean arterial pressure values measured in the three groups throughout the study. There were no significant differences between the baseline values of mean arterial pressure in the three groups. In control dogs, no significant change occurred at any time. Before coronary occlusion, enalaprilat and EXP3174 induced a decrease in mean arterial pressure which was reinforced by ischaemia. However, at the end of occlusion and during reperfusion, there were no significant differences in mean arterial pressure values between animals treated with saline, enalaprilat or EXP3174.

Regional myocardial blood flows

Table 2 shows regional myocardial blood flow values in the nonischaemic zones. These regional blood flows were not significantly different between the three groups at any time. However there was a small trend toward higher regional flows during occlusion and reperfusion in the animals treated with EXP3174 and during reperfusion in the animals treated with enalaprilat.

Table 3 shows regional myocardial blood flow values in the ischaemic zones. Coronary occlusion significantly reduced regional blood flows in the three groups. At reperfusion, regional blood flows returned to values that were not significantly different from the preocclusion ones, except in EXP3174-treated dogs were epicardial blood flow values were significantly greater ($P<0.05$). However, there was never any significant difference between regional myocardial blood flow values among the three groups of animals.

Infarct size

The size of area at risk, an important baseline predictor of infarct size, was similar in the three groups and averaged 38.2 ± 1.4% of the left ventricle in controls, 38.7 ± 1.6% in the enalaprilat-treated group, and 39.9 ± 1.1% in the EXP 3174-treated group (Figure 1). Analysis of infarct size (Figure 1), expressed either as a percentage of the left ventricle (14.5 ± 2.8% in the control group, 16.5 ± 3.0% in the enalaprilat-treated group, 20.1 ± 3.5% in the EXP3174-treated group) or as a percentage of the area at risk (36.9 ± 6.6% in the control group, 42.1 ± 7.0% in the enalaprilat-treated group, 49.8 ± 8.5% in the EXP3174-treated group) indicated no significant differences among the three groups. Thus, by direct group comparison, neither of the treatments was able to limit infarct size. Inasmuch as it is well known that variations in infarct size may be due to variations in collateral blood flow (Reimer *et al.*, 1985), we also investigated the relation between infarct size and collateral blood flow (Figure 2). There was a close inverse relation between infarct

Table 1 Heart rate and mean arterial pressure values at baseline, before ischaemia, 10 and 90 min into ischaemia and 60 min into reperfusion in the three groups of animals

	Group	*Baseline*	*Before ischaemia*	*10 min ischaemia*	*90 min ischaemia*	*60 min reperfusion*
Heart rate (beats min^{-1})	Saline	133 ± 7	133 ± 7	133 ± 5	129 ± 8	123 ± 6
	Enalaprilat	137 ± 8	132 ± 8	133 ± 8	131 ± 9	123 ± 9
	EXP3174	134 ± 4	128 ± 6	135 ± 5	140 ± 15	127 ± 7
Mean arterial pressure (mmHg)	Saline	89 ± 6	88 ± 6	83 ± 7	77 ± 7	76 ± 8
	Enalaprilat	96 ± 5	72 ± 6*	59 ± 4**	71 ± 7	73 ± 5
	EXP3174	86 ± 2	76 ± 4*	63 ± 4**	76 ± 6	75 ± 7

Values are mean ± s.e.mean.
Value significantly different (*$P<0.05$; **$P<0.01$) from corresponding saline value.

Table 2 Regional (Endo: endocardial, mid: middle, Epi: epicardial) and transmural (Trans) myocardial blood flows (ml min^{-1} g^{-1}) values in the nonischaemic zones before ischaemia, 10 min into ischaemia and 60 min into reperfusion in the three groups of animals

		Endo	*Mid*	*Epi*	*Trans*
Saline	Before ischaemia	0.69 ± 0.05	0.62 ± 0.05	0.57 ± 0.05	0.63 ± 0.05
	10 min ischaemia	0.82 ± 0.06	0.71 ± 0.05	0.66 ± 0.05	0.73 ± 0.06
	60 min reperfusion	0.76 ± 0.10	0.68 ± 0.09	0.62 ± 0.08	0.69 ± 0.09
Enalaprialt	Before ischaemia	0.89 ± 0.08	0.84 ± 0.08	0.77 ± 0.09	0.83 ± 0.08
	10 min ischaemia	0.85 ± 0.13	0.81 ± 0.13	0.79 ± 0.13	0.82 ± 0.13
	60 min reperfusion	0.98 ± 0.10	0.98 ± 0.11	0.98 ± 0.13	0.98 ± 0.11
EXP3174	Before ischaemia	0.76 ± 0.07	0.70 ± 0.07	0.66 ± 0.06	0.71 ± 0.07
	10 min ischaemia	0.93 ± 0.09	0.85 ± 0.08	0.84 ± 0.07	0.88 ± 0.08
	60 min reperfusion	1.00 ± 0.04	0.93 ± 0.03	0.89 ± 0.03	0.94 ± 0.03

Values are mean ± s.e.mean.

Table 3 Regional (Endo: endocardial, mid: middle, Epi: epicardial) and transmural (Trans) myocardial blood flows (ml min^{-1} g^{-1}) values in the ischaemic zones before ischaemia, at 10 min into ischaemia and at 60 min into reperfusion in the three groups of animals

		Endo	*Mid*	*Epi*	*Trans*
Saline	Before ischaemia	0.78 ± 0.08	0.71 ± 0.08	0.68 ± 0.10	0.73 ± 0.08
	10 min ischaemia	0.04 ± 0.01[ab]	0.08 ± 0.02[ab]	0.16 ± 0.03[ab]	0.09 ± 0.02[ab]
	60 min reperfusion	0.86 ± 0.16	0.85 ± 0.12	0.79 ± 0.13	0.84 ± 0.08
Enalaprialt	Before ischaemia	0.93 ± 0.07	0.87 ± 0.07	0.80 ± 0.07	0.87 ± 0.07
	10 min ischaemia	0.04 ± 0.02[ab]	0.08 ± 0.02[ab]	0.15 ± 0.03[ab]	0.09 ± 0.02[ab]
	60 min reperfusion	1.27 ± 0.36	1.17 ± 0.25	0.86 ± 0.12	1.09 ± 0.21
EXP3174	Before ischaemia	0.86 ± 0.08	0.82 ± 0.08	0.72 ± 0.08	0.79 ± 0.07
	10 min ischaemia	0.04 ± 0.02[ab]	0.06 ± 0.02[ab]	0.11 ± 0.03[ab]	0.06 ± 0.02[ab]
	60 min reperfusion	0.69 ± 0.19	0.96 ± 0.11	1.17 ± 0.08[ac]	0.93 ± 0.09

Values are mean ± s.e.mean.
[a]Value significantly different ($P<0.01$) from before occlusion value.
[b]Value significantly different ($P<0.01$) from 60 min reperfusion value.
[c]Value significantly different ($P<0.01$) from 60 min reperfusion value in saline- and enalaprilat-treated dogs.

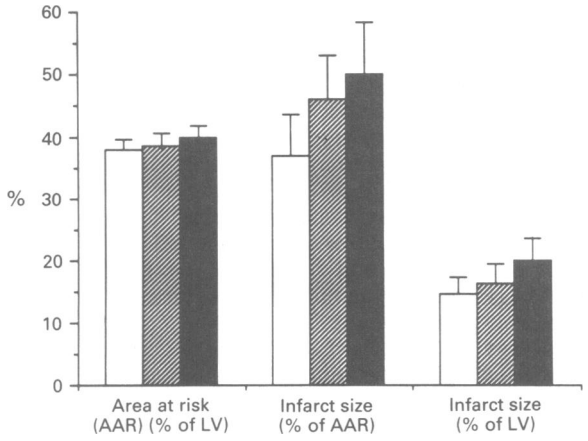

Figure 1 Area at risk and infarct size (expressed as percentage of area at risk and as percentage of left ventricle). Open columns, control; hatched columns, enalaprilat; solid columns, EXP3174.

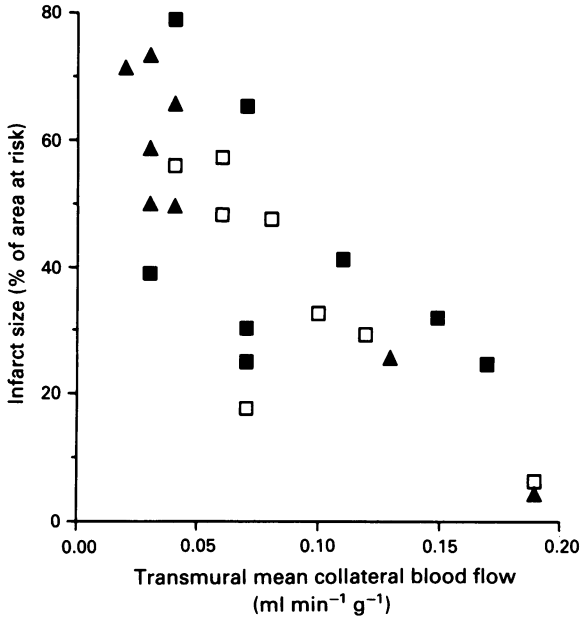

Figure 2 Plot of transmural collateral blood flow versus infarct size in saline (□)- enalaprilat (■)- and EXP3174 (▲)-treated dogs.

size and collateral blood flow in controls. Neither enalaprilat nor EXP3174 altered this relationship, suggesting that none of these treatments affected infarct size. This visual observation was confirmed by an analysis of covariance with collateral blood flow as an independent variable and infarct size as a dependent variable ($F = 0.35$; P = NS). Thus clearly neither of the treatments limited infarct size, even when the variation due to collateral flow was controlled.

Discussion

In this study, intravenous administration of enalaprilat induced a marked inhibition (91%) of the pressor response to angiotensin I, indicating an almost complete angiotensin-converting enzyme inhibition. Similarly, administration of EXP3174 resulted in a strong inhibition (87%) of the response to angiotensin I, demonstrating an almost complete blockade of the AT_1 receptor responsible for the pressor effects of angiotensin II, in agreement with the results of Wong *et al.* (1991) in conscious dogs. In addition, the fact that a similar blockade of angiotensin I pressor responses was achieved with EXP3174 and with enalaprilat indicates that the effects of the two drugs were investigated at an identical level of inhibition of the renin-angiotensin system.

The renin-angiotensin system plays a minor role in the regulation of mean arterial pressure (Laubie *et al.*, 1984) and coronary blood flow (Noguchi *et al.*, 1985; Gerard *et al.*, 1990) in normal dogs. Thus in this study this system was activated by a frusemide-pretreatment. As a result, EXP3174 and enalaprilat decreased mean arterial pressure whereas at the same doses and for the same level of inhibition of the renin-angiotensin system, these compounds do not exhibit any effect in non frusemide-pretreated dogs (Richard *et al.*, 1993). The decrease in mean arterial pressure induced by both EXP3174 and enalaprilat is related to the inhibition of the vasoconstrictor (Liang *et al.*, 1982) and sympathetic system facilitating (Clough *et al.*, 1982) properties of angiotensin II. This hypotension induced by both treatments was reinforced after 10 min of coronary occlusion, whereas in control dogs ischaemia did not induce any modification of mean arterial pressure. Thus, this result confirms the role of the renin-angiotensin system in the control of blood pressure in frusemide-treated dogs. In contrast, Ertl *et al.* (1983) reported that the renin-angiotensin system does not contribute importantly to the control of blood pressure following coronary artery occlusion. However, it must be stressed that the latter experiments were performed with saralasin, a peptide angiotensin II antagonist which exhibits a significant intrinsic partial agonist activity, mimicking the actions of angiotensin II itself (Chiu *et al.*, 1991).

In our experiments, the hypotension induced by either EXP3174 or enalaprilat was transient. At the end of the ischaemic period, no significant difference in arterial pressure was seen among the three groups. Comparison of the values obtained after 10 and 90 min of ischaemia revealed that this was due both to a decrease in blood pressure in controls and to an increase in blood pressure in treated animals. The reason for the increase in arterial pressure (despite persistent

blockade of the pressor response to angiotensin II) is not clear, but could be due to ischaemia-induced compensatory mechanisms (such as sympathetic stimulation), which could compete with the decrease in arterial pressure resulting from the inhibition of the renin-angiotensin system.

In our experimental model, no significant changes in regional myocardial blood flows were observed after administration of either the ACEI or the angiotensin II AT_1 antagonist. Furthermore, and despite the decrease in mean arterial pressure observed after 10 min of coronary artery occlusion, neither drug induced a decrease in collateral blood flow. The lack of effects of EXP3174 and enalaprilat on regional myocardial blood flows is in agreement with previous studies which showed (a) that these regional flows and their distribution (between epicardial and endocardial layers and between nonischaemic and iscahemic zones) are not modified by four ACEIs in anaesthetized dogs submitted to repeated coronary occlusions (Berdeaux *et al.*, 1987), and (b) that in anaesthetized open-chest dogs with or without activation of the renin-angiotensin system, no change in regional myocardial blood flow occurs after intravenous administration of enalaprilat and EXP3174 in nonischaemic conditions (Richard *et al.*, 1993). Thus our present data indicate that the renin-angiotensin system does not play a major role in the regulation of myocardial blood flows in the canine model of ischaemia-reperfusion in confirmation of what has been observed in the above mentioned studies.

The fact that neither enalaprilat nor EXP3174 limited infarct size in our study demonstrates that angiotensin II is not importantly involved in myocardial ischaemia-reperfusion injury. In this respect, our results are in agreement with those of other studies performed with ACEIs (Liang *et al.*, 1982; Daniell *et al.*, 1984; De Lorgeril *et al.*, 1992). In contrast, other investigators have reported an increase in regional myocardial blood flows and a limitation of infarct size with captopril in dogs (Ertl *et al.*, 1982). However, several experiments suggest that the anti-ischaemic effect of the sulphydryl-containing ACEI captopril could be due to non specific effects of this drug, such as an inhibition of oxygen free radical-dependent reperfusion injury (Westlin & Mullane, 1988; Chopra *et al.*, 1992) and/or an interference with arachidonic acid metabolism (Van Gilst *et al.*, 1987). However, in some experimental models of ischaemia, acute administration of non sulphydryl-containing ACEIs also limited infarct size in rats (Hock *et al.*, 1985) and in cats (Lefer & Peck, 1984). Differences in animal models, duration and severity of ischaemia, occurrence of reperfusion, and drug concentrations could contribute to these conflicting results. Finally it must be stressed that exclusion of dogs from the study because of the development of intractable ventricular fibrillation did not lead to an underestimation of infarct size in the control group as only one of the excluded animals belonged to that group.

Several experiments have suggested that the anti-ischaemic effects of non-sulphydryl containing ACEIs could entirely be related to their capacity to potentiate the cardioprotective effects of bradykinin (Becker *et al.*, 1989; Martorana *et al.*, 1990; Van Gilst *et al.*, 1992; Baumgarten *et al.*, 1993). For example, Martorana *et al.* (1990) have shown that the infarct size-limiting effect of ramipril in a dog model of 6 h permanent ischaemia was abolished by a bradykinin antagonist. However, our experiments do not support this hypothesis since enalaprilat did not induce any limitation of infarct size. The discrepancies between the results of Martorana *et al.* (1990) and ours could be explained by differences in the models used. In the former study, experiments were performed in non-salt depleted dogs, i.e., a situation of low renin-angiotensin system activation. Also, in that study, the ACEI was administered into the coronary artery, thus avoiding the systemic effects of the drug. Finally, it must be stressed that Martorana *et al.* (1990) did not measure collateral blood flow. Thus, it is possible that the observed beneficial effect of ramipril in this preparation was due to undetected differences in the level of collateral blood flow between the different experimental groups.

In conclusion, neither EXP3174 nor enalaprilat limited infarct size in frusemide-pretreated dogs. Thus, it appears that the renin-angiotensin system does not significantly contribute to myocyte death in the canine ischaemia-reperfusion model.

This work was supported by a joint grant from the Institut National de la Santé et de la Recherche Médicale (INSERM) and Merck, Sharp and Dohme Laboratories (91 AN 21). The authors wish to thank Alain Bizé for his excellent technical assistance. B.G. was a recipient of a fellowship grant from the Fondation de la Recherche Médicale.

References

AMBROSIONI, E. & BORGHI, C. (1989). Potential use of ACE inhibitors after acute myocardial infarction. *J. Cardiovasc. Pharmacol.*, **14** (Suppl. 9), S92–S94.

BAUMGARTEN, C.R., LINZ, W., KUNKEL, G. SCHOLKENS, B.A. & WIEMER, G. (1993). Ramiprilat increases bradykinin outflow from isolated hearts of rat. *Br. J. Pharmacol.*, **108**, 293–295.

BECKER, R.H.A., LINZ, W. & SCHOLKENS, B.A. (1989). Pharmacological interference with the cardiac renin-angiotensin system. *J. Cardiovasc. Pharmacol.*, **14** (Suppl. 4), S10–S15.

BERDEAUX, A., BONHENRY, C. & GIUDICELLI, J.F. (1987). Effects of four angiotensin I converting enzyme inhibitors on regional myocardial blood flow and ischaemic injury during coronary artery occlusion in dogs. *Fundam. Clin. Pharmacol.*, **1**, 201–212.

CHOPRA, M., BESWICK, H., CLAPPERTON, M., DARGIE, H.J., SMITH, W.E. & MCMURRARY, J. (1992). Antioxidant effects of angiotensin-converting enzyme (ACE) inhibitors, free radical and oxidant scavenging are sulfhydryl dependent, but lipid peroxidation is inhibited by both sulfhydryl- and nonsulfhydryl-containing ACE inhibitors. *J. Cardiovasc. Pharmacol.*, **19**, 330–340.

CHIU, A.T., MCCALL, D.E., PRICE, W.A., Jr., WONG, P.C., CARINI, D.J., DUNCIA, J.V., WEXLER, R.R., YOO, S.E., JOHNSON, A.L. & TIMMERMANS, P.B.M.W.M. (1991). In vitro pharmacology of DuP 753. *Am. Heart J.*, **4**, 282S–287S.

CLOUGH, D.P., COLLIS, M.G., CONWAY, J., HATTON, R. & KEDDIE, J.R. (1982). Interaction of angiotensin-converting enzyme inhibitors with the function of the sympathetic nervous system. *Am. J. Cardiol.*, **49**, 1410–1414.

DANIELL, H.B., CARSON, R.R., BALLARD, K.D., THOMAS, G.R. & PRIVITERA, P.J. (1984). Effects of captopril in limiting infarct size in conscious dogs. *J. Cardiovasc. Pharmacol.*, **6**, 1043–1047.

DE LORGERIL, M., OVIZE, M., DELAYE, J. & RENAUD, S. (1992). Importance of the flow perfusion deficit in the response to captopril in experimental myocardial infarction. *J. Cardiovasc. Pharmacol.*, **19**, 324–329.

ERTLE, G. (1987). Coronary vasoconstriction in experimental myocardial ischaemia. *J. Cardiovasc. Pharmacol.*, **9** (Suppl. 2), S9–S17.

ERTL, G., ALEXANDER, R.W. & KLONER, R.A. (1983). Interactions between coronary occlusion and the renin-angiotensin system in the dog. *Basic Res. Cardiol.*, **78**, 518–533.

ERTL, G., KLONER, R.A., ALEXANDER, R.W. & BRAUNWALD, E. (1982). Limitation of experimental infarct size by an angiotensin-converting enzyme inhibitor. *Circulation*, **65**, 40–48.

GERARD, J.L., PUSSARD, E., BERDEAUX, A. & GIUDICELLI, J.F. (1990). Haemodynamic and cardiac effects of spiraprilat in normal and sodium depleted conscious dogs. *Fundam. Clin. Pharmacol.*, **4**, 547–558.

GROVER, G.J., SLEPH, P.G., DZWONCZYK, S., WANG, P., FUNG, W., TOBIAS, D. & CUSHMAN, D.W. (1991). Effects of different angiotensin-converting enzyme (ACE) inhibitors on ischaemic isolated rat hearts, relation between cardiac ACE inhibition and cardioprotection. *J. Pharmacol. Exp. Ther.*, **257**, 919–929.

HEUSCH, G. (1990). α-adrenergic mechanisms in myocardial ischaemia. *Circulation*, **81**, 1–13.

HEYNDRICKX, G.R., BOETTCHER, D.H. & VATNER, S.F. (1976). Effects of angiotensin, vasopressin and methoxamine on cardiac function and blood flow distribution in conscious dogs. *Am. J. Physiol.*, **231**, 1579–1587.

HOCK, C.E., RIBEIRO, L.G.T. & LEFER, A.M. (1985). Preservation of ischaemic myocardium by a new converting enzyme inhibitor, enalaprilic acid, in acute myocardial infarction. *Am. Heart J.*, **109**, 222–228.

KOBAYASHI, M., FURUKAWA, Y. & CHIBA, S. (1978). Positive chronotropic and inotropic effects of angiotensin II in the dog heart. *Eur. J. Pharmacol.*, **50**, 17–25.

LAUBIE, M., SCHIAVI, P., VINCENT, M. & SCHMITT, H. (1984). Inhibition of angiotensin I-converting enzyme with S 949, biochemical effects, interspecies differences, and role of sodium diet in haemodynamic effects. *J. Cardiovasc. Pharmacol.*, **6**, 1076–1082.

LEFER, A.M. & PECK, R.C. (1984). Cardioprotective effects of enalapril in acute myocardial ischaemia. *Pharmacology*, **29**, 61–69.

LIANG, C., GAVRAS, H., BLACK, J., SHERMAN, L.G. & HOOD, W.B.Jr. (1982). Renin-angiotensin system inhibition in acute myocardial infarction in dogs. *Circulation*, **66**, 1249–1255.

LINDER, C. & HEUSCH, G. (1990). ACE inhibitors for the treatment of myocardial ischaemia? *Cardiovasc. Drugs Ther.*, **4**, 1375–1384.

MARTORANA, P.A., KETTENBACH, B., BRIEPOHL, G., LINZ, W. & SCHOLKENS, B.A. (1990). Reduction of infarct size by local angiotensin-converting enzyme inhibition is abolished by a bradykinin antagonist. *Eur. J. Pharmacol.*, **182**, 395–396.

NOGUCHI, K., KATO, T., ITO, H., ANIYA, Y. & SAKANASHI, M. (1985). Effect of intracoronary captopril on coronary blood flow and regional myocardial function in dogs. *Eur. J. Pharmacol.*, **110**, 11–19.

PRZYKLENK, K. & KLONER, R.A. (1989). Relationships between structure and effects of ACE inhibitors, comparative effects in myocardial ischaemia/reperfusion injury. *Br. J. Clin. Pharmacol.*, **28**, 167S–175S.

REIMER, K.A., JENNINGS, R.B., COBB, F.R., MURDOCK, R.H., GREENFIELD, J.C. Jr., BECKER, L.C., BULKLEY, B.J., HUTCHINS, G.M., SCHWARTZ, R.P. Jr., BAILEY, K.R. & PASSAMANI, E.R. (1985). Animal models for protecting ischaemic myocardium, results of the NHLBI cooperative study. Comparison of unconscious and conscious dog models. *Circ. Res.*, **56**, 651–665.

RICHARD, V., BERDEAUX, A. & GIUDICELLI, J.F. (1993). Systemic and coronary effects of the angiotensin II receptor antagonist EXP3174 in dogs. *J. Cardiovasc. Pharmacol.*, **22**, 52–57.

SCHOMIG, A., DART, A.M., DIETZ, R., MAYER, E. & KUBLER, W. (1984). Release of endogenous catecholamines in the ischaemic myocardium of the rat. Part A, locally mediated release. *Circ. Res.*, **55**, 689–701.

VAN GILST, W.H., TIO, R.A., VAN WIJNGAARDEN, J., DE GRAEFF, P.A. & WESSELING, H. (1992). Effects of converting enzyme inhibitors on coronary flow and myocardial ischaemia. *J. Cardiovasc. Pharmacol.*, **19** (Suppl. 5), S134–S139.

VAN GILST, W.H., VAN WIJNGAARDEN, J., SCHOLTENS, E., DE GRAEF, P.A., DE LANGEN, C.D.J. & WESSELING, H. (1987). Captopril-induced increase in coronary flow, an SH-dependent effect on arachidonic acid metabolism? *J. Cardiovasc. Pharmacol.*, **9** (Suppl. 2), S31–S36.

WESTLIN, W. & MULLANE, K. (1988). Does captopril attenuate reperfusion-induced myocardial dysfunction by scavenging free radicals? *Circulation*, **77** (Suppl. 1), 30–39.

WONG, P.C., HART, S.D., DUNCIA, J.V. & TIMMERMANS, P.B.M. W.M. (1991). Nonpeptide angiotensin II receptor antagonists. Studies with DuP 753 and EXP3174 in dogs. *Eur. J. Pharmacol.*, **202**, 323–330.

WONG, P.C., PRICE, W.A. Jr., CHIU, A.T., DUNCIA, J.V., CARINI, D.J., WEXLER, R.R., JOHNSON, A.L. & TIMMERMANS, P.B.M.W.M. (1990). Nonpeptide angiotenin II receptor antagonists. XI. Pharmacology of EXP3174, an active metabolite of DuP 753, an orally active antihypertensive agent. *J. Pharmacol. Exp. Ther.*, **255**, 211–217.

(Received May 2, 1993
Revised June 1, 1993
Accepted July 1, 1993)