Effect of enalaprilat on bradykinin and des-Arg⁹-bradykinin release following reperfusion of the ischaemic rat heart

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1 The release of bradykinin (BK) and its metabolite, des-Arg⁹-bradykinin (des-Arg⁹-BK), was studied following reperfusion of a globally ischaemic rat heart.

2 BK-like immunoreactivity increased from 13 ± 3 (preischaemic value) to 48 ± 12 fmol min⁻¹ g⁻¹ ($P < 0.05$, $n = 14$) 30 s after reperfusion. No difference in BK release was found between control hearts and hearts pretreated with the angiotensin converting enzyme (ACE or kininase II) inhibitor, enalaprilat (50 ng ml⁻¹).

3 No significant change in des-Arg⁹-BK-like immunoreactivity during reperfusion was observed in control hearts. In contrast, des-Arg⁹-BK-like immunoreactivity rose from 44 ± 15 to 177 ± 61 fmol min⁻¹ g⁻¹ ($P < 0.05$, $n = 7$) 30 s after reperfusion in enalaprilat-treated hearts.

4 In conclusion, BK is released upon reperfusion of the globally ischaemic rat heart. ACE inhibitors, through the inhibition of kininase II, increase the formation of the active metabolite, des-Arg⁹-BK.

Keywords: Bradykinin; des-Arg⁹-bradykinin; ischaemia; reperfusion; angiotensin-converting enzyme inhibitors; rat heart

Introduction

Angiotensin converting enzyme (ACE), also known as kininase II, is responsible for the conversion of angiotensin I to angiotensin II. ACE is also involved in the catabolism of kinins. ACE inhibitors have been shown to reduce infarct size (Hartman *et al.*, 1993) and to improve ventricular function (Linz *et al.*, 1992; Massoudy *et al.*, 1994) following myocardial ischaemia and reperfusion. The reversal by the bradykinin (BK) receptor antagonist, Hoe 140, of the protective effects of ACE inhibitors suggests an important contribution of kinins in the cardioprotection afforded by these drugs.

Studies reporting changes in BK formation during ischaemia and reperfusion are sparse. It has been recently reported that ischaemia is accompanied by an increase in BK-like immunoreactivity in the coronary effluent (Baumgarten *et al.*, 1993). The ischaemia-induced BK release was enhanced in the presence of the ACE inhibitor, ramiprilat (Baumgarten *et al.*, 1993). BK is also a substrate for kininase I, which yields the biologically active metabolite, des-Arg⁹-bradykinin (des-Arg⁹-BK). However, the effect of ischaemia on des-Arg⁹-BK formation has not been documented until now. The aim of this study was therefore to evaluate simultaneously the release of both BK and des-Arg⁹-BK following ischaemia and reperfusion, in the presence or absence of an ACE inhibitor.

Methods

Preparation of hearts

The investigation was performed in accordance with the Canadian Council on Animal Care. Male Sprague-Dawley rats (300–350 g) were narcotised by a short exposure (10–20 s) to a 100% CO₂ atmosphere and killed by decapitation. The thorax was rapidly opened and the heart excised and immersed into ice-cold buffer containing heparin (20 iu ml⁻¹). Hearts were then mounted on the experimental setup and perfused at constant flow (10 ml min⁻¹) by means of a digital roller pump (Masterflex Microprocessor Pump Drive, Cole Parmer Instruments Co. Chicago, IL, U.S.A.). A 20-ml compliance chamber along the perfusion line ensured a continuous flow. The perfusion solution consisted of a modified Krebs-Henseleit

buffer containing (mmol l⁻¹): NaCl 118, KCl 4, CaCl₂ 2.5, KH₂PO₄ 1.2, MgSO₄ 1, NaHCO₃ 24, D-glucose 5, and pyruvate 2. The perfusate was gassed with 95% O₂: 5% CO₂ (pH 7.4) and kept at a constant temperature of 37°C. Enalaprilat or its vehicle (water) were administered through a Y-connector in the aortic cannula with a syringe pump (Harvard Model 11 Microprocessor Syringe Pump, England) at 1/100th of the coronary flow rate. Adequate mixing of the drug was ensured by the turbulent flow created in the reverse-drop shaped aortic cannula. Coronary perfusion pressure was measured with a pressure transducer (Cobe, model CDX3) connected to a sidearm of the aortic perfusion cannula. Iso-volumetric left ventricular pressure and its first derivative (dP/dt) was measured by a fluid-filled latex balloon (Size no. 4, Hugo Sachs Elektronik, March-Hugstetten, Germany) inserted into the left ventricle through a pulmonary vein and connected to a second pressure transducer. The volume of the balloon was adjusted to obtain a diastolic pressure between 5 and 10 mmHg. Heart rate was derived from the left ventricular pressure signal. Data were recorded on a Grass Model 79 polygraph system.

Experimental protocol

After a 15-min stabilization period, perfusion of enalaprilat (50 ng ml⁻¹) or its vehicle was started, and the hearts were left to stabilize for a further 15 min. The hearts were then exposed to a 20-min zero-flow ischaemia, followed by a 10 min reperfusion. Ischaemia was induced by turning off perfusion pumps. Conversely, restarting both roller (Krebs-Henseleit buffer) and syringe (enalaprilat or vehicle) pumps to their pre-ischaemic flow rates ensured reperfusion. During the ischaemic period, hearts were immersed into Krebs-Henseleit buffer kept at 37°C. Collection of the coronary effluent for the assay of kinin-like immunoreactivity was performed prior to ischaemia, and during the whole reperfusion period, between the following time-points: 0–1, 1–3, 3–6 and 6–10 min of reperfusion.

Assay of kinin-like immunoreactivity

BK- and des-Arg⁹-BK-like immunoreactivity was assayed in the coronary effluent. For this purpose, the perfusate was

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collected into tubes containing ice-cold trifluoroacetic acid (0.05 ml of a 10% solution per ml of perfusate) under constant agitation. The perfusate was then cleaned by chromatography on C₈-silica column (Waters, Milford MA, U.S.A.) as described earlier (Décarié *et al.*, 1994; Raymond *et al.*, 1995). The eluate containing kinins was evaporated to dryness in a Speed Vac system (Savant, Farmingdale, NJ, U.S.A.). The residue was dissolved in 400 µl of a Tris-HCl buffer (50 mmol l⁻¹) containing Tween 20 (0.5 ml l⁻¹) and NaCl (100 mmol l⁻¹). BK (Décarié *et al.*, 1994) and des-Arg⁹-BK (Raymond *et al.*, 1995) were quantified using a specific chemiluminescence enzyme immunoassay as described earlier. These assays use highly specific polyclonal immunoglobulins induced in rabbit against the carboxyterminal structure of BK and des-Arg⁹-BK, respectively. Both assays are characterized by a sensitivity level of 0.1 fmol ml⁻¹ (BK) and 27 fmol ml⁻¹ (des-Arg⁹-BK), respectively.

Statistical analysis

All data are presented as a mean value ± s.e.mean. Single values in control and treated hearts were compared by Student's unpaired *t* test. Bradykinin and des-Arg⁹-BK release upon reperfusion was analysed using a 2 by 5 factorial analysis of variance. Probability less than 0.05 was considered to be statistically significant.

Results

The mean weight of control (*n*=7) and enalaprilat-treated (*n*=7) hearts was 1.22 ± 0.03 and 1.26 ± 0.05 g, respectively. At a constant flow rate of 10 ml min⁻¹, coronary perfusion pressure measured prior to ischaemia was 70 ± 9 and 67 ± 7 mmHg for control and enalaprilat-treated hearts, respectively (Table 1). Values of heart rate, left ventricular developed pressure (peak systolic pressure minus diastolic pressure), and *dP/dt* were also comparable between control and enalaprilat-treated hearts (Table 1).

During ischaemia, all hearts stopped beating and left ventricular diastolic pressure rose to 43 ± 9 and 35 ± 8 mmHg in control and enalaprilat-treated hearts, respectively. Following reperfusion, coronary perfusion pressure returned promptly to preischaemic values (65 ± 2 and 64 ± 4 mmHg for control and enalaprilat-treated hearts, respectively). Left ventricular diastolic pressure rose further, reaching values at the end of the reperfusion period of 67 ± 6 and 55 ± 7 mmHg for control and enalaprilat-treated hearts, respectively. Only one control (out of 7) and one enalaprilat-treated (out of 7) heart exhibited a complete and stable recovery upon reperfusion, with left ventricular *dP/dt* above 1200 mmHg s⁻¹: the others showed either no recovery, or transient periods of recovery that were interrupted by events compatible with either rapid ventricular tachycardia or fibrillation. Left ventricular developed pressure and *dP/dt* values at the end of the reperfusion period were 15 ± 3 mmHg and 521 ± 143 mmHg s⁻¹ for control, and 26 ± 11 mmHg and 579 ± 222 mmHg s⁻¹ for enalaprilat-treated hearts, respectively.

Table 1 Haemodynamic variable values before ischaemia

	Control (<i>n</i> =7)	Enalaprilat (<i>n</i> =7)
Coronary perfusion pressure (mmHg)	70 ± 9	67 ± 7
Heart rate (beats min ⁻¹)	293 ± 16	288 ± 8
Left ventricular developed pressure (mmHg)	101 ± 9	100 ± 10
Maximum left <i>dP/dt</i> (mmHg s ⁻¹)	2114 ± 105	2257 ± 211

A rapid and transient increase in BK-like immunoreactivity (from 16 ± 5 to 55 ± 19 fmol min⁻¹ g⁻¹, *P* < 0.05, *n*=7) was observed 30 s after reperfusion in control hearts (Figure 1). A statistically comparable increase was found in enalaprilat-treated hearts (from 11 ± 3 to 41 ± 16 fmol min⁻¹ g⁻¹,

Figure 1 Bradykinin-like immunoreactivity measured in the coronary effluent before ischaemia and following reperfusion of globally ischaemic rat hearts in the absence (○) and presence (●) of 50 ng ml⁻¹ enalaprilat. **P* < 0.05, compared to the corresponding preischaemic (0 min) value.

Figure 2 Des-Arg⁹-bradykinin-like immunoreactivity measured in the coronary effluent before ischaemia and following reperfusion of globally ischaemic rat hearts in the absence (○) and presence (●) of 50 ng ml⁻¹ enalaprilat. **P* < 0.05, ***P* < 0.01, compared to the corresponding preischaemic (0 min) value. #*P* < 0.05, compared to the corresponding value in control hearts.

$P < 0.05$, $n = 7$). No increase in des-Arg⁹-BK-like immunoreactivity was observed in control hearts (Figure 2). In contrast, a marked increase in des-Arg⁹-BK-like immunoreactivity (from 44 ± 15 to 177 ± 61 fmol min⁻¹ g⁻¹, $P < 0.05$, $n = 7$) was observed 30 s after reperfusion in enalaprilat-treated hearts (Figure 2).

Discussion

In the present study, we have observed a marked release of BK upon reperfusion of the globally ischaemic rat heart. In contrast, release of des-Arg⁹-BK upon reperfusion was observed only in the presence of enalaprilat.

A recent study has reported an increase in BK outflow during regional ischaemia produced by coronary artery ligation in the rat isolated heart (Baumgarten *et al.*, 1993). In contrast to our study, BK outflow fell upon reperfusion. The increased BK outflow during the ligation period, with no further increase upon reperfusion, suggested that BK originated from normal tissue surrounding the ischaemic myocardium. In our study, the whole heart was made ischaemic. Furthermore, BK peaked in the first fraction collected, and declined thereafter. Therefore, in our experimental conditions, it is most likely that BK originated from, and accumulated in, the ischaemic myocardium, being washed away upon reperfusion. It should be noted that, in the study by Baumgarten *et al.* (1993), BK release was expressed relative to the right ventricle in the ischaemic period and to the whole heart for the reperfusion.

Pretreatment of hearts with the ACE inhibitor, enalaprilat, did not enhance the release of BK upon reperfusion. This is in contrast with the study by Baumgarten *et al.* (1993) in which a more important BK outflow during ischaemia was observed in the presence of ACE inhibition. The absence of enhanced BK release in the presence of enalaprilat suggests that metabolic pathways distinct from kininase II can play an important role in the catabolism of BK in our experimental model. For example, kininase II inhibition with the ACE inhibitor could shunt BK towards other catabolic pathways such as kininase I. This hypothesis is supported by the huge release of des-Arg⁹-BK in enalaprilat-treated hearts.

Baumgarten *et al.* (1993) measured a concentration of immunoreactive kinins in the ng ml⁻¹ g⁻¹ range. This is in contrast with our results, which are in the pg ml⁻¹ g⁻¹ range. This discrepancy could be explained by analytical differences. The

presence of kinin precursors and/or kinin-forming enzymes in the perfusate could interfere with the assay. In the present study, the efficacy of the extraction step and the specificity of the quantification method have been carefully validated (Dcarie *et al.*, 1994; Raymond *et al.*, 1995).

Our study is the first report of a major release of des-Arg⁹-BK upon reperfusion. Des-Arg⁹-BK is a natural agonist for the B₁ kinin receptor (Marceau & Regoli, 1991). This receptor is believed to be expressed in different pathological states (Marceau & Regoli, 1991). However, a constitutive B₁ kinin receptor has been described recently in the dog *in vivo* (Nakhostine *et al.*, 1993). Pretreatment of hearts with des-Arg⁹-BK prior to global ischaemia has been found to reduce the incidence of ventricular fibrillation and noradrenaline outflow (Chahine *et al.*, 1993), as well as atrial natriuretic peptide release (Lvesque Rouleau *et al.*, 1994), upon reperfusion of the ischaemic rat heart. In the present study, enalaprilat failed to improve ventricular functional recovery upon reperfusion, despite the increase in endogenous des-Arg⁹-BK formation. The experimental conditions in the present study are slightly different from the earlier work. Therefore, direct comparison may be perilous. Furthermore, the mean concentration of endogenous des-Arg⁹-BK measured in the coronary effluent was 2.3×10^{-11} mol l⁻¹, which is low compared to the concentration (10^{-7} mol l⁻¹) of exogenous des-Arg⁹-BK used to achieve cardioprotection (Chahine *et al.*, 1993). However, the fate of exogenous des-Arg⁹-BK administered in such conditions, and thus the concentration reaching the myocytes, is unknown and will be the subject of future studies.

In conclusion, BK is released upon reperfusion of the globally ischaemic rat heart. ACE inhibitors, through the inhibition of kininase II, increase the formation of the active metabolite, des-Arg⁹-BK. Further studies are needed to assess the role of des-Arg⁹-BK in the cardioprotective effect of ACE inhibitors.

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