Ramiprilat increases bradykinin outflow from isolated hearts of rat

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To establish that bradykinin is formed in the heart we measured bradykinin in the venous effluent from rat isolated hearts perfused with Krebs-Henseleit buffer. In addition, we examined the effect on bradykinin outflow of the angiotensin converting enzyme (ACE) inhibitor, ramiprilat. From rat isolated normoxic hearts a bradykinin outflow of $0.85 \pm 0.1 \text{ ng ml}^{-1}$ perfusate g^{-1} wet weight was measured. Perfusion with ramiprilat increased the bradykinin concentration to $2.8 \pm 0.3 \text{ ng ml}^{-1}$ perfusate g^{-1} wet weight. During ischaemia bradykinin outflow maximally increased 8.2 fold to $7.0 \pm 0.5 \text{ ng ml}^{-1}$ perfusate g^{-1} , and in ramiprilat-perfused hearts 5.8 fold to $16.0 \pm 1.8 \text{ ng ml}^{-1}$ perfusate g^{-1} . In the reperfusion period bradykinin outflow normalized to values measured in the respective pre-ischaemic period.

The presents data show that bradykinin is continuously formed in the rat isolated heart. Ischaemia increases bradykinin outflow from the heart. Presumably by inhibiting degradation of kinins, ACE inhibition significantly increased the bradykinin concentration during normoxia, ischaemia and reperfusion.

Keywords: Bradykinin-formation; rat isolated hearts; ischaemia; angiotensin converting enzyme (ACE)-inhibition; ramiprilat

Introduction Angiotensin converting enzyme (ACE) inhibitors like ramiprilat act not only through inhibition of formation of angiotensin (AII) but also through inhibition of bradykinin degradation. Recent data from rat isolated working hearts showed that both ramiprilat and bradykinin improve myocardial metabolism during ischaemia and this was accompanied by a reduction in reperfusion injuries (Linz *et al.*, 1990). ACE inhibition and bradykinin perfusion evoked identical beneficial changes in cardiodynamics and metabolism in these hearts. The protective effects were abolished by the specific BK_2 bradykinin receptor antagonist Hoe 140 suggesting that bradykinin might be beneficial in myocardial ischaemia (Linz *et al.*, 1990). In the present study, we were interested in whether bradykinin is formed locally in rat isolated hearts. Therefore we measured bradykinin release in the venous effluent of rat isolated ischaemic hearts perfused with Krebs-Henseleit buffer, with and without the ACE inhibitor, ramiprilat.

Methods Male Wistar rats (weighing 280–300 g) Møllegaard, Skensved, Denmark) were given sodium heparin (500 ug kg^{-1}) intraperitoneally (i.p.) 1 h before the animals were anaesthetized. Anaesthesia was induced by i.p. injection of 200 mg kg^{-1} hexobarbitone (Evipan). The hearts were excised and placed in ice-cold perfusion medium until contraction had ceased after approximately 5 s. Isolated hearts were perfused via the aorta at a constant perfusion pressure equivalent to 65 mmHg with Krebs-Henseleit solution gassed with 95% O_2 plus 5% CO_2 at 37°C and pH 7.4. The perfusate did not recirculate. Hearts were perfused with and without ramiprilat $1 \times 10^{-7} \text{ mol l}^{-1}$ for an initial 15-min control perfusion period. Then, acute regional myocardial ischaemia was produced by occlusion of the left coronary artery close to its origin. Arterial occlusion was maintained for 15 min after which the occlusion was removed and arterial reperfusion commenced.

One ml of the venous effluent was directly collected into EDTA-solution ($10^{-4} \text{ mol l}^{-1}$) after 5 and 10 min in the control perfusion period (pre-ischaemic period), after 5, 7.5, 10 and 15 min in the ischaemic period, and 1, 2, 3 and 4 min after reperfusion period. Subsequently the samples were lyophilized and resuspended in 0.2 M Tris/0.01 M EDTA/0.1% lysozyme, pH 6.4. Bradykinin concentrations were measured by use of a specific radioimmunoassay capable of detecting 20 pg ml^{-1} of kinin (Proud *et al.*, 1983). Bradykinin concentrations were expressed in ng ml^{-1} perfusate related to g wet weight of the whole heart ($792 \pm 160 \text{ mg}$) in the control and reperfusion period and of the right ventricle ($132 \pm 15 \text{ mg}$) in the ischaemic period. This procedure is valid because the rat heart has been shown to have few collaterals (Schaper *et al.*, 1988).

Statistical analysis was performed with ANOVA followed by the Bonferroni test when appropriate. Differences were considered significant if $P < 0.05$. Results are given as mean $\pm$ s.e.mean.

Results In the control period, basal coronary flow in Krebs-Henseleit buffer perfused hearts was $13.9 \pm 0.7 \text{ ml min}^{-1}$. During ischaemia, coronary flow was reduced by about 50% ($7.6 \pm 0.9 \text{ ml min}^{-1}$) and recovered in the reperfusion period ($15.3 \pm 1.1 \text{ ml min}^{-1}$). In Krebs-Henseleit buffer perfused hearts, basal bradykinin outflow in the control period was $0.85 \pm 0.1 \text{ ng ml}^{-1}$ perfusate g^{-1} wet weight after 10 min perfusion, increased during the ischaemic period to $7.0 \pm 0.5 \text{ ng ml}^{-1}$ perfusate g^{-1} wet weight after 7.5 min perfusion, and immediately returned to initial values in the reperfusion period (Figure 1).

In the presence of the ACE inhibitor, ramiprilat, coronary flow was not significantly changed when compared to Krebs-Henseleit buffer-perfused hearts. The respective values were $13.7 \pm 0.6 \text{ ml min}^{-1}$ (control period), $7.8 \pm 1.0 \text{ ml min}^{-1}$ (ischaemic period) and $14.1 \pm 1.1 \text{ ml min}^{-1}$ (reperfusion period). However, addition of the ACE inhibitor, ramiprilat, significantly enhanced the bradykinin concentrations in all three perfusion periods. In the control period the concentration of bradykinin in the venous effluent increased to

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Figure 1 Bradykinin release from rat isolated hearts: (○) Krebs-Henseleit buffer-perfused hearts; (●) ramiprilat (1×10^{-7} mol l^{-1})-perfused hearts. Each value represents mean $\pm$ s.e.mean (vertical bars) of 12 control hearts and 12 hearts perfused with ramiprilat. * $P < 0.05$ vs Krebs-Henseleit buffer-perfused hearts (controls).

2.8 ± 0.3 ng ml^{-1} perfusate g^{-1} wet weight after 10 min perfusion. This was a 3.2 fold increase when compared with the Krebs-Henseleit buffer-perfused control group. In the ischaemic period, local ACE inhibition with ramiprilat induced a maximum increase of the bradykinin concentration in the venous effluent of 16.0 ± 1.8 ng ml^{-1} perfusate g^{-1} wet weight after 7.5 min perfusion, being back to initial values in the reperfusion period (Figure 1).

Discussion The present data show that bradykinin is released from rat isolated hearts perfused with Krebs-Henseleit buffer. This basal bradykinin release was increased 3.2 fold when the hearts were perfused with the ACE inhibitor ramiprilat, dissolved in Krebs-Henseleit solution. In comparison with normoxic controls, ischaemia increased bradykinin release 8.2 fold and 5.8 fold in the absence and presence of ramiprilat, respectively whereas in the reperfusion period in both groups, bradykinin release reached the values of the respective control perfusion period within 1 min. The missed overshoot of kinin release in the early reperfusion phase could be explained by the fact that the stimulus of ischaemia had been removed. Furthermore oxygen free radicals which occur in the early reperfusion phase might be responsible for an accelerated breakdown of kinins.

Increased bradykinin levels during ischaemia were observed by Wilkens *et al.* (1970) who reported that a small fall

in cardiac tissue pH, which follows an ischaemic insult, induces an activation of the local kinin system. Hashimoto *et al.* (1977) found an increased concentration of bradykinin in coronary sinus blood after coronary occlusion in the dog. Also in the dog, myocardial ischaemia induced by coronary artery stenosis and sympathetic stimulation caused the heart to release kinins (Matsuki *et al.*, 1987). And lastly in man, kinin levels in peripheral blood were found to increase soon after myocardial infarction, which led Hashimoto *et al.* (1978) to suggest that kinins released in patients with infarction may have a compensatory cardioprotective effect.

Our own experiments on rat isolated hearts with post-ischaemic reperfusion injuries (Linz *et al.*, 1990) and dog experiments with myocardial infarction (Martorana *et al.*, 1991) support this hypothesis. With the BK_2 -bradykinin receptor antagonist, Hoe 140, all observed bradykinin- or ramiprilat-mediated effects on the heart could be abolished (Linz *et al.*, 1990; Martorana *et al.*, 1991). Even in rats with aortic banding developing hypertension and left ventricular hypertrophy, the antihypertrophic effect of ramiprilat could be reversed by Hoe 140 (Linz & Schölkens, 1992).

A further argument for bradykinin-mediated actions of ACE inhibitors is the fact that ACE activity but also gene expression is increased during myocardial ischaemia as well as left ventricular hypertrophy (Schunkert *et al.*, 1990; Johnston *et al.*, 1991). Inhibition of local ACE (kininase II)-activity by ACE inhibitors reduces AII formation and bradykinin metabolism. In this way the difference in bradykinin release of Krebs-Henseleit buffer versus ramiprilat-perfused hearts is explained by the inhibition of the local ACE activity in the heart when the ACE inhibitor is perfused.

Preliminary studies where endothelium was destroyed by H_2O_2 perfusion showed that bradykinin release in Krebs-Henseleit buffer and ramiprilat-perfused hearts was almost abolished indicating that bradykinin released from the heart mainly comes from endothelial cells (data not shown). This agrees with the results of Wiemer *et al.* (1991) showing that ramiprilat was capable of inhibiting the breakdown of endothelium-derived bradykinin in human cultured and bovine aortic endothelial cells.

Based on earlier findings with bradykinin and ACE inhibitors and on the present results with direct measurement of bradykinin in the venous effluent from rat isolated hearts perfused with Krebs-Henseleit buffer with or without ramiprilat, we suggest that ischaemia activates proteases in the heart resulting in continuous bradykinin generation. Furthermore local ACE inhibition in these isolated hearts reduces bradykinin breakdown with subsequent increased bradykinin outflow which is accompanied by an amelioration of ischaemia-reperfusion-induced injuries, including suppression of reperfusion arrhythmias (Linz *et al.*, 1990).

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