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Role of kinins in the endothelial protective effect of ischaemic preconditioning

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1 The aim of this study was to assess whether the protective effect of ischaemic preconditioning on endothelial function in coronary arteries of the rat involves kinins.

2 Isolated hearts of the rat were exposed to a 30-min low-flow ischaemia (flow rate of 1 ml min^{-1}) followed by 20-min reperfusion, after which coronaries were precontracted with 0.1 μM U-46619, and the response to the endothelium-dependent vasodilator, 5-hydroxytryptamine (5-HT, 10 μM), compared to that of the endothelium-independent vasodilator, sodium nitroprusside (SNP, 3 μM).

3 In untreated hearts, ischaemia-reperfusion diminished selectively 5-HT-induced vasodilatation, compared with time-matched sham hearts. The vasodilatation to SNP was unaffected after ischaemia-reperfusion. Preconditioning (5 min of zero-flow ischaemia followed by 10 min reperfusion) in untreated hearts preserved the vasodilatation produced by 5-HT.

4 Blockade of B_1 and B_2 receptors with either 3 nM [Lys^0, Leu^8, des-Arg^9]-bradykinin (LLDBK) or 10 nM Hoe 140 (icatibant), respectively, (started 15 min before ischaemic preconditioning or a corresponding sham period and stopped just before the 20-min reperfusion period) had no effect on the vasodilatation produced by either 5-HT or SNP in sham hearts. Pretreatment with Hoe 140 did not block the protective effect of ischaemic preconditioning on the 5-HT vasodilatation. In contrast, LLDBK halved the protective effect of ischaemic preconditioning on endothelium-dependent vasodilatation.

5 Perfusion with either bradykinin or des-Arg^9-bradykinin (1 nM) 30 min before and lasting throughout the ischaemia protected the endothelium.

6 In conclusion, ischaemic preconditioning affords protection to the endothelial function in coronary resistance arteries of the rat partly by activation of B_1 receptors. Although exogenous BK perfusion can protect the endothelium, B_2 receptors do not play an important role in this protection in the rat isolated heart.

Keywords: Coronary circulation; endothelium; ischaemic preconditioning; kinins

Introduction

Ischaemic preconditioning induced by single or repetitive short periods of ischaemia followed by intermittent reperfusion, renders the heart more resistant to a subsequent longer ischaemic period. This phenomenon limits infarct size (Murry *et al*., 1986; Cohen *et al*., 1991), reduces the risk of ischaemia-reperfusion arrhythmias (Shiki & Hearse, 1987; Hagar *et al*., 1991), improves recovery of ventricular function (Cohen *et al*., 1991), reduces catabolite accumulation and slows ischaemic metabolism (Murry *et al*., 1990; Reimer *et al*., 1994). This cardioprotective effect has been observed in different species, including rats (Li & Kloner, 1992; Yellon *et al*., 1992), rabbits (Cohen *et al*., 1991), dogs (Murry *et al*., 1986), pigs (Sack *et al*., 1993) and man (Yellon *et al*., 1993).

Some studies have demonstrated that ischaemia-reperfusion attenuated endothelial function in large coronary vessels (Van Benthuysen *et al*., 1987; Pearson *et al*., 1990) and in coronary microvessels (DeFily & Chilian, 1993). In addition, it has been shown that the beneficial effect of ischaemic preconditioning is not limited to the cardiomyocytes, but can be observed in endothelial cells in various experimental models including dog resistance coronary arteries *in vivo* (DeFily & Chilian, 1993), and coronary circulation of the rat *in vitro* (Richard *et al*., 1994; Bouchard & Lamontagne, 1996). Adenosine (Miura & Iimura, 1993; Liu *et al*., 1994; Bouchard & Lamontagne, 1996), ATP sensitive potassium channels (K_{ATP} channels) (Grover, 1994; Parratt & Kane, 1994; Bouchard & Lamontagne, 1996), and protein kinase C activation (Mitchell *et al*., 1995) have been implicated in the mechanisms of the protection afforded by ischaemic preconditioning.

The contribution of kinins in the protection afforded by ischaemic preconditioning has been studied in the rat isolated heart as well as in dogs and rabbits *in vivo*. These studies revealed that B_2-receptor activation is involved in the reduction in both ischaemia and reperfusion-induced arrhythmias (Vegh *et al*., 1994) and infarct size (Wall *et al*., 1994; Goto *et al*., 1995), and in the improvement of post-ischaemic ventricular recovery by ischaemic preconditioning (Brew *et al*., 1995). Numerous studies have demonstrated that exogenous bradykinin perfusion is protective against ischaemia-reperfusion induced ventricular fibrillation (Linz *et al*., 1990; Vegh *et al*., 1991). In addition, it has been shown that exogenous perfusion of bradykinin (BK) and des-Arg^9-bradykinin (DBK) decreased reperfusion-induced noradrenaline outflow and arrhythmias via B_1-receptors (Chahine *et al*., 1993).

However, little is known about the role played by endogenous kinins in the endothelial protective effect of ischaemic preconditioning. Therefore, the initial aim of the present study was to evaluate whether ischaemic preconditioning affords protection against the ischaemic insult to the endothelium of coronary vessels in rat isolated hearts via the kinin pathway. The second aim was to verify whether exogenous kinin perfusion could mimic the beneficial effects of ischaemic preconditioning against ischaemic insult in these hearts.

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Methods

Preparation of hearts

The investigation was performed in accordance with the guidelines from the Canadian Council on Animal Care. Male Sprague-Dawley rats (300–350 g) were narcotized with CO_2 until a complete loss of consciousness and rapidly decapitated. Hearts were rapidly excised and immersed in ice-cold heparin-treated buffer (10 iu ml^{-1}). They were immediately mounted on the Langendorff setup and perfused at constant flow by means of a digital roller pump. A 20-ml compliance chamber along the perfusion line ensured a continuous flow. The flow rate was adjusted during the stabilization period to obtain a coronary perfusion pressure of approximately 75 mmHg and was held constant, with the exception of the ischaemic periods during which flow was either stopped (zero-flow ischaemia) or reduced to 1 ml min^{-1} (low-flow ischaemia). A second adjustment of the flow rate was made at the end of the long reperfusion period, before the perfusion of U-46619, to correct any deviation of the coronary perfusion pressure from 75 mmHg, and was held constant thereafter. Flow rate was measured during the whole experiment with an in-line ultrasonic flow probe and meter (Transonic Systems Inc., model T106). Perfusion pressure was monitored to calculate coronary resistance. The normal perfusion solution consisted of a modified Krebs-Henseleit buffer containing (in mM): NaCl 118, KCl 4, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1, $NaHCO_3$ 24, D-glucose 5 and pyruvate 2. The perfusate was gassed with 95% O_2-5% CO_2 (pH 7.4) and kept at a constant temperature of 37°C. All drugs were administered through a Y connector in the aortic cannula with syringe pumps (Harvard Apparatus, model 11) at one hundredth of the coronary flow rate. Adequate mixing of the drugs was ensured by the turbulent flow created in the reverse drop shaped aortic cannula. All concentrations mentioned in the text and figures refer to the final concentration after mixing. Coronary perfusion pressure was measured with a pressure transducer connected to a side arm of the aortic perfusion cannula. Isovolumetric left ventricular pressure and its first derivative (dP/dt) was measured by a fluid filled latex balloon inserted into the left ventricle 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 trace by a tachograph. Data were recorded on a polygraph system (Grass Model 79 polygraph).

Experimental protocols

The animals were placed randomly into fifteen groups (Figure 1). The hearts in all groups were subjected to a 20-min stabilization period. Drugs or vehicle infusion was then started, followed by an additional 15-min perfusion period. The ischaemic groups were subjected to a 15-min sham period, followed by 30 min of partial ischaemia (flow rate 1 ml min^{-1}) before a 20-min reperfusion period. In the preconditioned groups, the hearts were exposed to 5 min global ischaemia (zero-flow) plus 10 min of reperfusion before the 30-min ischaemia and 20-min reperfusion periods. The sham groups were not exposed to ischaemia-reperfusion, but to a time-matched normal perfusion. After these periods, coronary arteries were precontracted with 0.1 μM U-46619 administered throughout the end of the experiment. Fifteen minutes after the beginning of U-46619 infusion, the endothelial function was evaluated by the vasodilatation produced by 10 μM 5-hydroxytryptamine (5-HT), whereas coronary smooth muscle function was evaluated with 3 μM sodium nitroprusside (SNP). These infusions were maintained for 10 min, which was long enough to reach a steady state. A washout period of 10 min was allowed between each infusion. Vasodilatation was evaluated by computing % changes in coronary resistance (coronary perfusion pressure divided by coronary flow), measured immediately before each drug infusion, and after a new steady state. The concentrations of 5-HT and SNP were determined in preliminary dose-response experiments to produce near-maximal vasodilatation.

Sham, ischaemic, and ischaemic preconditioned hearts were treated with either 10 nM Hoe 140 (icatibant, a selective B_2 receptor antagonist), 3 nM [Lys^0, Leu^8, des-Arg^9]-bradykinin (LLDBK, a selective B_1 receptor antagonist), or vehicles

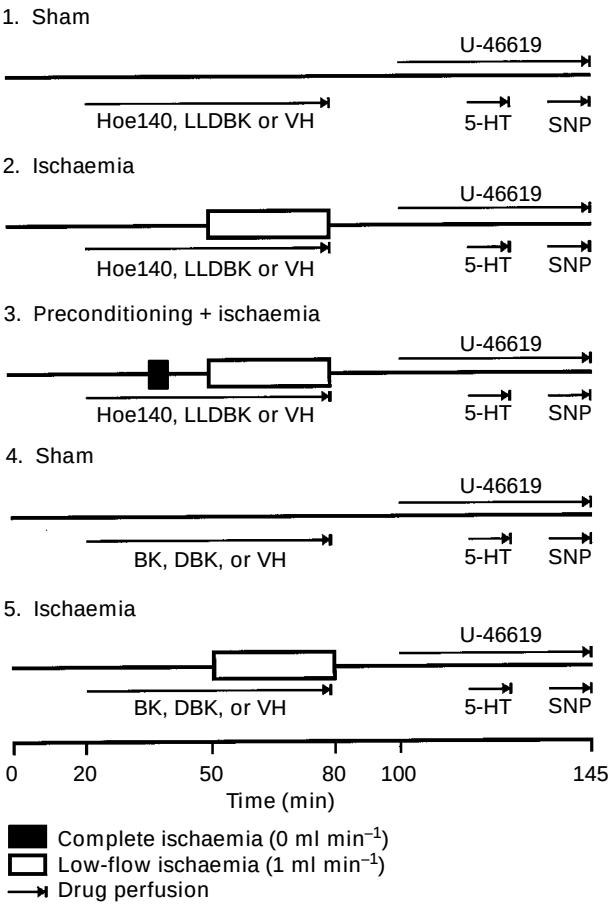

Figure 1 Diagrams showing the different experimental protocols. Each experiment started with a 20-min stabilization period. Hearts in the ischaemia protocol (no. 2 and 5) underwent 30 min of low-flow (1 ml min^{-1}) ischaemia and 20 min of reperfusion, after an additional 30 min stabilization period. Hearts in the ischaemic preconditioning + ischaemia protocol (no. 3) were submitted to a preconditioning 5 min zero-flow ischaemia and 10 min reperfusion, before the 30-min low-flow ischaemia. Perfusion of drugs (Hoe 140 10 nM (no. 1, 2 and 3), [Lys^0,Leu^8,des-Arg^9]-bradykinin 3 nM (LLDBK, no. 1, 2 and 3), bradykinin 1 nM (BK, no. 4 and 5), des-Arg^9-bradykinin 1 nM (DBK, no. 4 and 5), or their respective vehicles (VH)) was started after the 20-min stabilization period, lasted throughout the 30-min low-flow ischaemia or a corresponding period, and was stopped upon reperfusion. In all protocols, endothelial and smooth muscle function was evaluated after the 20-min reperfusion period. Coronary arteries were precontracted by a continuous infusion of 0.1 μM U-46619. After 15 min, infusion of 5-HT (10 μM) was started for 10 min. A wash-out period of 10 min was allowed between 5-HT and sodium nitroprusside (SNP, 3 μM, 10 min) infusions. The bottom axis represents the time in min.

starting after the 20-min stabilization period, and lasting throughout the 30-min partial ischaemic period. Drug infusion was stopped upon reperfusion.

In additional experimental series, the effect of an exogenous kinin perfusion was compared with that of ischaemic preconditioning. In these groups, hearts were treated with either 1 nM BK, 1 nM DBK or vehicle starting after the 20-min stabilization period, in order to expose the hearts to 30 min kinin perfusion before ischaemia. The kinin perfusion lasted throughout the 30-min ischaemic period, and was stopped upon reperfusion.

Statistical analysis

Values represent the mean ± s.e.mean. Statistical significance of differences between means was evaluated by a two way analysis of variance followed by a Scheffé *post-hoc* test. In the presence of an interaction between the different groups, one way analysis of variance were used for each group. A commercially available software (Systat for Windows version 6.1) was used. Only probability values (P) smaller than 0.05 were considered to be statistically significant.

Drugs

Hoe 140 was kindly provided by Hoechst-Marion-Roussel (Frankfurt, Germany). LLDBK was a generous gift from Dr A. Adam. All other drugs were obtained from Sigma-Aldrich (Mississauga, Ont, Canada). Stock solutions of Hoe 140 (0.1 mM), LLDBK (1 mM), BK (0.9 mM), and DBK (1.12 mM) were prepared in Krebs-Henseleit buffer, and further diluted in the same buffer. U-46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$, 28.5 mM) was dissolved in 100% ethanol and diluted in Krebs-Henseleit buffer to obtain the desired final concentration. Ethanol (0.003%) at the concentration obtained in the final dilution had no effect on any of the haemodynamic variables studied and on the dilator responses to 5-HT and SNP. All the other drugs were dissolved in Krebs-Henseleit buffer.

Results

Ischaemic preconditioning groups

Vascular function

Untreated groups Coronary resistance measured just before 0.1 μM U-46619 perfusion ($n=24$) was 5.92 ± 0.29 mmHg min ml^{-1}, for a coronary flow rate of 6.72 ± 0.22 ml min^{-1} g^{-1} (mean heart weight of 1.90 ± 0.05 g). Infusion of U-46619 (0.1 μM, $n=24$) induced a significant ($P<0.05$) vasoconstriction in all groups of hearts (sham, ischaemia, and ischaemic preconditioning, Table 1). Perfusion of 10 μM 5-HT produced a diminution in coronary resistance of −25.2 ± 4.8%. Thirty minutes of partial ischaemia significantly diminished the 5-HT induced vasodilatation by more than half (Figure 2a). One period of ischaemic preconditioning prevented the deleterious effect of ischaemia on endothelium-dependent vasodilatation: the vasodilatation produced by 5-HT in preconditioned hearts was comparable to that observed in hearts not subjected to ischaemia (Figure 2a). Endothelium-independent vasodilatation to 3 μM SNP was not affected by ischaemia and was found to be comparable in the three groups (sham, ischaemia and ischaemic preconditioning, Figure 2b).

Table 1 Effect of 0.1 μM U-46619 infusion on coronary resistance (mmHg min ml^{-1})

	n	*Before U-46619*	*After U-46619*
Ischaemic preconditioning groups			
Untreated			
Sham	8	5.87 ± 0.35	10.10 ± 0.82 *
Ischaemia	8	5.96 ± 0.82	11.53 ± 0.75 *
Preconditioning	8	5.94 ± 0.31	11.53 ± 0.75 *
Hoe 140			
Sham	7	7.58 ± 0.60	13.15 ± 0.87 *
Ischaemia	7	8.21 ± 1.22	12.28 ± 0.60 *
Preconditioning	13	6.16 ± 0.31	10.63 ± 1.37 *
LLDBK			
Sham	5	6.71 ± 0.61	11.33 ± 0.68 *
Ischaemia	6	4.64 ± 0.58	9.06 ± 1.43 *
Preconditioning	6	7.18 ± 0.55	12.05 ± 0.62 *
Exogenous kinin groups			
Bradykinin			
Sham	7	7.53 ± 0.74	11.90 ± 0.72 *
Ischaemia	5	5.52 ± 0.20	10.00 ± 0.61 *
Ischaemia without BK	8	5.94 ± 0.31	11.53 ± 0.75 *
Des-Arg^9 Bradykinin			
Sham	6	6.02 ± 0.60	10.51 ± 0.72 *
Ischaemia	6	5.45 ± 0.55	10.41 ± 1.27 *
Ischaemia without DBK	8	5.94 ± 0.31	11.53 ± 0.75 *

Coronary resistance was calculated as perfusion pressure (mmHg) divided by perfusion flow (ml min^{-1}). Values are means ± s.e.mean. * $P<0.05$ compared with the corresponding before U-46619 value.

Hoe 140 treated groups Blockade of kinin B_2-receptors with Hoe 140 (10 nM) was accompanied by a small but non-significant increase in coronary resistance when measured just before 0.1 μM U-46619 perfusion (Hoe 140 treated vs untreated hearts, $P>0.05$, Table 1). The perfusion rate was 5.57 ± 0.32 ml min^{-1} g^{-1} (mean heart weight of 2.18 ± 0.06 g). Infusion of U-46619 (0.1 μM, $n=27$) induced a significant ($P<0.05$) vasoconstriction in all Hoe 140 treated groups (Table 1). Vasodilatation produced by 10 μM 5-HT (−20.3 ± 4.2% in sham hearts, $n=7$) was almost totally abolished in the ischaemic group (Figure 2c). Ischaemic preconditioning in Hoe 140 treated hearts was still able to prevent completely the deleterious effect of ischaemia on 5-HT-induced vasodilatation (Figure 2c). Vasodilatation to 3 μM SNP was comparable in the three Hoe 140 treated groups (sham, ischaemia and ischaemic preconditioning, Figure 2d).

LLDBK treated groups Blockade of kinin B_1 receptors with LLDBK produced no significant increase in coronary resistance when measured just before 0.1 μM U-46619 perfusion (LLDBK treated vs untreated hearts, $P>0.05$, Table 1). The perfusion rate was 5.75 ± 0.31 ml min^{-1} g^{-1} (mean heart weight of 2.16 ± 0.08 g). Infusion of U-46619 (0.1 μM, $n=17$) induced a significant ($P<0.05$) vasoconstriction in all three groups (Table 1). Vasodilatation produced by 10 μM 5-HT (−35.7 ± 4.5% in sham hearts, $n=5$) was practically abolished in the ischaemic group (Figure 2e). Ischaemic preconditioning in LLDBK treated hearts was unable to prevent the deleterious effect of ischaemia on 5-HT-induced vasodilatation (Figure 2e). Vasodilatation to 3 μM SNP was comparable in the three LLDBK treated groups (sham, ischaemia and ischaemic preconditioning, Figure 2f).

Myocardial function The inotropic and lusitropic characteristics of Hoe 140 and LLDBK pretreated hearts were

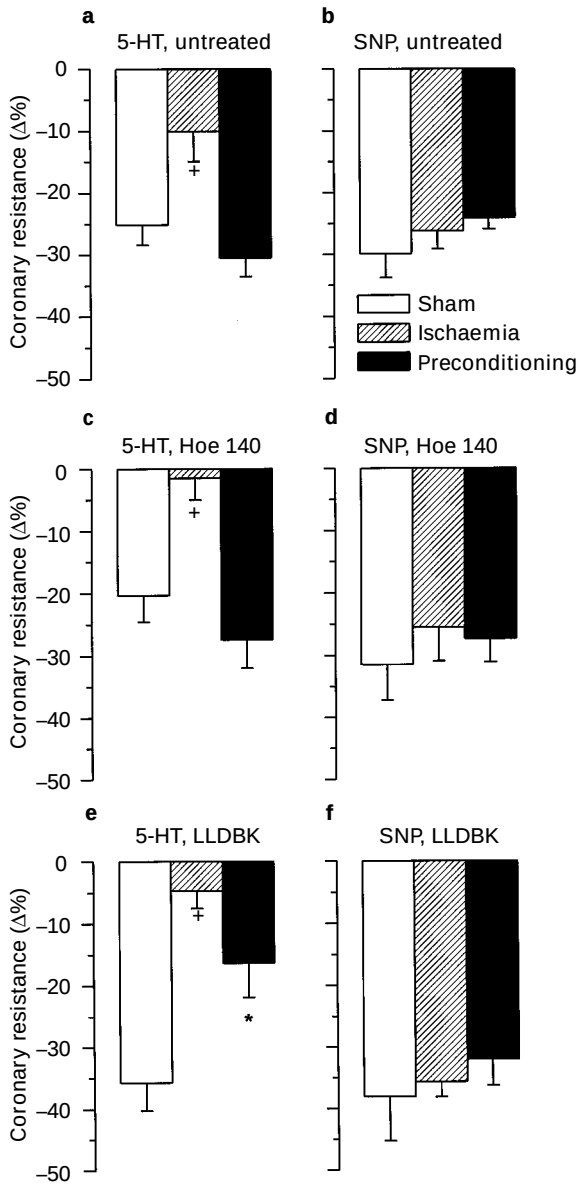

Figure 2 Change in coronary resistance (Δ%) induced by 10 μM 5-HT (a, c and e) and 3 μM sodium nitroprusside (SNP, b, d and f) in control hearts (a and b), in Hoe 140-pretreated hearts (c and d), and in [Lys^0,Leu^8,des-Arg^9]-bradykinin (LLDBK) pretreated hearts (e and f). Number of sham, ischaemic and ischaemic preconditioning hearts were 8, 8 and 8 for untreated; 7, 7 and 13 for Hoe 140; and 5, 6, and 6 for LLDBK pretreated hearts, respectively. †$P<0.05$, compared with sham and ischaemic preconditioning. *$P<0.05$, compared with sham.

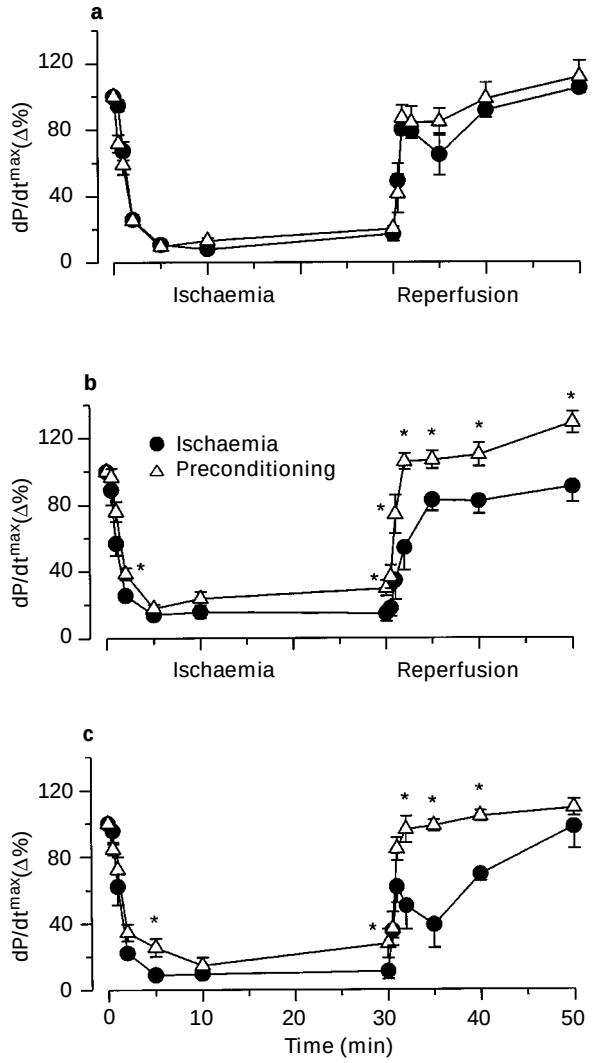

Figure 3 Change in dP/dt$_{max}$ (Δ%) observed during 30 min low-flow ischaemia (1 ml min^{-1}) and 20 min reperfusion in untreated (a), and in Hoe 140 (10 nM) (b) and [Lys^0,Leu^8,des-Arg^9]-bradykinin (LLDBK, 3 nM) (c) pretreated hearts. Number of ischaemic and ischaemic preconditioning hearts were 8 and 8 for untreated; 7 and 13 for Hoe 140; and 6 and 6 for LLDBK pretreated hearts, respectively. *$P<0.05$ compared with ischaemic hearts.

comparable to those of untreated hearts: dP/dt$_{max}$ values measured before the 30-min low-flow ischaemia were 1980±449 ($n=16$), 1453±45 ($n=20$) and 1532±104 mmHg s^{-1} ($n=12$), and the dP/dt$_{min}$ values 934±120 ($n=16$), 966±42 ($n=20$) and 1041±65 mmHg s^{-1} ($n=12$), for untreated, Hoe 140 and LLDBK pretreated hearts, respectively. Low-flow ischaemia was accompanied by a severe reduction in dP/dt$_{max}$ (Figure 3) and in dP/dt$_{min}$ (Figure 4) in all groups. Ischaemic preconditioning improved dP/dt$_{max}$ and dP/dt$_{min}$ recovery during ischaemia, particularly during the last 20 min of ischaemia, in LLDBK pretreated (Figures 3 and 4) and Hoe 140 pretreated (Figures 3 and 4), but not in untreated hearts. Ischaemic preconditioning improved the post-ischaemic recovery of dP/dt$_{max}$ (Figure 3) and dP/dt$_{min}$ (Figure 4) in LLDBK pretreated (Figures 3 and 4) and Hoe 140 pretreated (Figures 3 and 4), but not in untreated hearts (Figures 3 and 4).

Exogenous kinins groups

Vascular function

BK treated groups Perfusion with BK (1 nM) produced no significant effect on coronary resistance when measured just before the 30-min ischaemic period (0.7±2.7% variation of coronary resistance, $n=12$). The perfusion rate in BK treated hearts was 6.11±0.32 ml min^{-1} g^{-1} (mean heart weight of 2.14±0.14 g). Infusion of U-46619 (0.1 μM, $n=12$) induced a significant ($P<0.05$) vasoconstriction in all BK treated hearts (Table 1). Thirty minutes of low-flow ischaemia significantly diminished the 5-HT-induced vasodilatation by more than half in untreated hearts (Figure 5a). Treatment with BK, starting 30 min before ischaemia, preserved the vasodilatation produced by 10 μM 5-HT in ischaemic hearts (Figure 5a). Vaso-

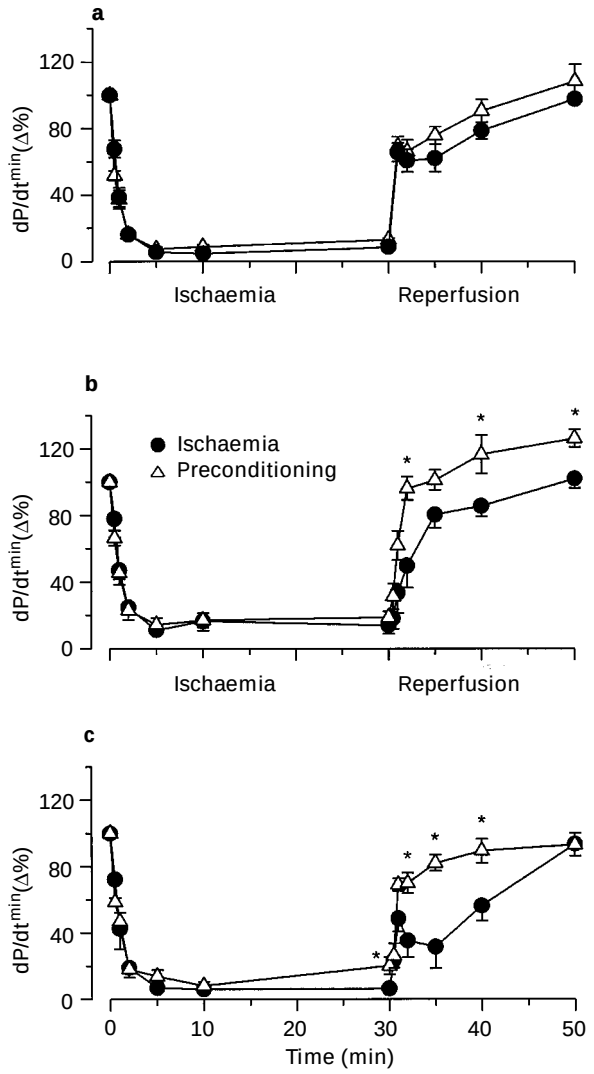

Figure 4 Change in dP/dt_{min} (Δ%) observed during 30 min low-flow ischaemia (1 ml min^{-1}) and 20 min reperfusion in untreated (a), and in Hoe 140 (10 nM) (b) and [Lys^0,Leu^8,des-Arg^9]-bradykinin (3 nM, LLDBK) (c) pretreated hearts. Number of ischaemic and ischaemic preconditioning hearts were 8 and 8 for untreated; 7 and 13 for Hoe 140; and 6 and 6 for LLDBK pretreated hearts, respectively. $*P<0.05$ compared with ischaemic hearts.

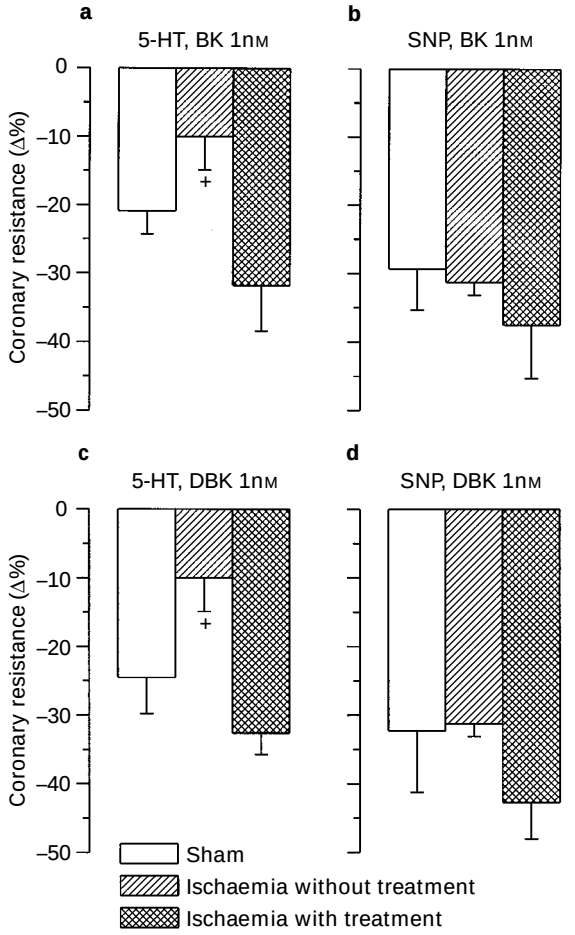

Figure 5 Change in coronary resistance (Δ%) induced by 10 μM 5-HT (a and c) and 3 μM sodium nitroprusside (SNP, b and d) in bradykinin (BK) pretreated hearts (a and b), and in des-Arg^9-bradykinin (DBK) pretreated hearts (c and d). Number of sham, ischaemia without treatment, and ischaemia with treatment hearts were 7, 8, and 5 for BK; and 6, 8, and 6 for DBK pretreated hearts, respectively. $\dagger P<0.05$, compared with sham and ischaemia with treatment.

dilatation to 3 μM SNP was comparable in all BK treated hearts (Figure 5b).

DBK treated groups DBK perfusion (1 nM) produced no significant effect on coronary resistance when measured just before the 30-min ischaemic period (3.5±3.0% variation of coronary resistance, $n=12$). The perfusion rate in DBK treated hearts was 6.41±0.54 ml min^{-1} g^{-1} (mean heart weight of 2.14±0.13 g). Infusion of U-46619 (0.1 μM, $n=12$) induced a significant ($P<0.05$) vasoconstriction in all DBK-treated hearts (Table 1). Thirty minutes of low-flow ischaemia halved the 5-HT-induced vasodilatation in untreated hearts (Figure 5c). Treatment with DBK, starting 30 min before ischaemia, preserved the vasodilatation produced by 10 μM 5-HT in ischaemic hearts (Figure 5c). Vasodilatation to 3 μM SNP was comparable in all DBK treated hearts (Figure 5d).

Myocardial function The dP/dt_{max} values measured before the 30-min low-flow ischaemia were 1483±71 and 1600±63 mmHg s^{-1}, and the dP/dt_{min} values 942±88 and 1180±116 mmHg s^{-1}, for BK 1 nM and DBK 1 nM pretreated hearts, respectively. In both groups, pretreatment with exogenous kinins, either DBK or BK 30-min before ischaemia, had no effect on ischaemic or post-ischaemic recovery of dP/dt_{max} and dP/dt_{min} (data not shown).

Discussion

In the present study, the contribution of kinins in the protective effect of ischaemic preconditioning on myocardial and endothelial functions of the rat heart was assessed. The major findings are (1) that ischaemic preconditioning with a single short period of ischaemia prevents endothelial dysfunction produced by ischaemia-reperfusion in rat hearts via activation of B_1 receptors, but not B_2 receptors, and (2) both DBK and BK perfusions starting 30 min before ischaemia can mimic the beneficial effect of ischaemic preconditioning on endothelial function in rat coronary arteries.

Effect of preconditioning on ischaemic dysfunction

In the present study, the ischaemic conditions (flow rate and duration) were selected in order to observe a selective

endothelial dysfunction. This was confirmed by the fact that the endothelium-dependent and NO-mediated (Mankad *et al.* 1991) vasodilatation of coronary arteries to 5-HT was drastically decreased after the ischaemia-reperfusion insult, whereas the same vessels retained the ability to dilate to SNP, an endothelium-independent vasodilator. It was demonstrated earlier (Bouchard & Lamontagne, 1996) that ischaemic preconditioning prevents the reduction in the vasodilatation to 5-HT after ischaemia-reperfusion, suggesting that ischaemic preconditioning can protect endothelial function in coronary arteries. Such a protection was observed in the present study. A protective effect of ischaemic preconditioning was also observed with canine epicardial coronary arteries (DeFily & Chilian, 1993), and in rat isolated left coronary arteries *in vitro* (Richard *et al.*, 1994).

The upper and lower limits of the dP/dt signal, dP/dt_{max} and dP/dt_{min}, which represent the capacity of the ventricle to contract during systole and its ability to relax during diastole, were used to evaluate the contractile function of the hearts. These variables recovered rapidly and completely within the 20-min reperfusion period. Furthermore, ischaemic preconditioning failed to improve post-ischaemic ventricular recovery. The inability of ischaemic preconditioning to improve ventricular recovery is most probably due to the fact that the present ischaemic conditions were too mild to depress severely the contractile function, leaving little room for improvement.

Role of B_2 receptors in ischaemic preconditioning

BK has often been shown to be an endogenous mediator of the protection afforded by ischaemic preconditioning (Parratt *et al.*, 1995). Some investigators have demonstrated that the B_2 receptor antagonist, Hoe 140, prevents the protective effect of ischaemic preconditioning on ventricular arrhythmias in dogs *in vivo* (Vegh *et al.*, 1994), on infarct size in rabbits *in vivo* (Wall *et al.*, 1994), and contractile function in isolated rat hearts (Brew *et al.*, 1995). However, to the best of our knowledge, little is known about the role played by endogenous kinins in the endothelial protective effect of ischaemic preconditioning. Therefore, we tested whether B_2 receptor activation was involved in the protection afforded by ischaemic preconditioning to the endothelium. Hoe 140 was chosen for its high selectivity for B_2 receptors. Furthermore, the concentration used was above those corresponding to the pA_2 values reported in the literature (Regoli *et al.*, 1996). The protective effect of ischaemic preconditioning on endothelial function was not affected by Hoe 140, as reflected by the comparable endothelium-dependent vasodilatation to 5-HT in both Hoe 140-treated preconditioned and sham groups. Therefore, these data suggest that B_2 receptor activation is not involved in the protection afforded by ischaemic preconditioning, against endothelial dysfunction observed following ischaemia-reperfusion.

In the present experimental conditions, Hoe 140 pretreatment seems to improve myocardial recovery in the preconditioned group, but this may rather be due to a slightly depressed myocardial recovery observed in the Hoe 140 treated ischaemic group. Thus, these data suggest that B_2 receptor activation may be required for a full post-ischaemic ventricular recovery under the present conditions.

Role of B_1 receptors in ischaemic preconditioning

Recently, it has been shown that the protective effect against ischaemia obtained by either BK or DBK perfusion is mediated via the activation of B_1 receptors (Chahine *et al.*, 1993). However, at present, the role played by B_1 receptor activation in the protective action of ischaemic preconditioning remains unknown. Therefore, we tested, using a potent and highly selective B_1 receptor antagonist (Gobeil *et al.*, 1996), whether the activation of B_1 receptors is involved in ischaemic preconditioning. In the LLDBK treated preconditioned group, the vasodilatation to 5-HT was halved whereas the vasodilatation to SNP was not significantly different from that observed in the sham group. These data suggest that an endogenously produced B_1-receptor agonist plays a role in the endothelial protection afforded by ischaemic preconditioning.

In the present experimental conditions, myocardial recovery was improved in the LLDBK treated preconditioned group, compared with the LLDBK treated ischaemic group. Once again, this phenomenon is probably due to the depressed myocardial recovery observed in the LLDBK treated ischaemic group, and suggests that B_1 receptor activation may also be needed for rapid post-ischaemic ventricular recovery.

Protective effect of exogenous kinins

To confirm the contribution of kinins in the endothelial protection afforded by ischaemic preconditioning, the effect of exogenous perfusion with low concentration of kinins on the endothelial function following ischaemia-reperfusion was studied. Two kinins were compared: BK and DBK, both were perfused 30 min before ischaemia. Both kinins prevented the ischaemia-induced reduction in the vasodilatation to 5-HT. Thus, these data suggest that both BK and DBK can mimic the protective effect of ischaemic preconditioning on the endothelial function.

In contrast to the endothelial function, neither BK nor DBK were able to improve ischaemic and post-ischaemic recovery of dP/dt_{max} and dP/dt_{min}. As discussed earlier, this is probably due to the fact that a 30-min 1 ml min^{-1} ischaemia, although sufficient to alter the endothelial function, is not severe enough to impair ventricular recovery.

The mechanisms by which kinins could exert a protective effect are numerous (Parratt *et al.*, 1995). Bradykinin, released from ischaemic tissues can act directly on B_2 receptors or can be metabolized into DBK and act on B_1 receptors (Linz *et al.*, 1996). We have recently observed that the protective effect of BK on the endothelial function in the rat isolated heart can be blocked by LLDBK (unpublished observation), suggesting that B_1 receptors might be implicated in the protective effect of BK. B_1 receptors could be expressed during the ischaemia as an induction of B_1 receptors has been found to occur in pathological conditions such as anoxia (Marceau & Regoli, 1991). Activation of B_2 and possibly B_1 receptors could stimulate the endothelial production of NO and prostacyclin both of which have been linked to K_{ATP} channel activation (Bouchard *et al.*, 1994; Miyoshi *et al.*, 1994). It has been recently demonstrated that inhibition of K_{ATP} channels with glibenclamide reduces the endothelial protection afforded by ischaemic preconditioning (Bouchard & Lamontagne, 1996). The exact mechanisms by which K_{ATP} channel activation protects the endothelium remain unknown, but several hypotheses have been proposed (Bouchard & Lamontagne, 1996).

Alternatively, both B_1 and B_2 receptors activate phospholipase C to release inositol 1,4,5-trisphosphate and 1,2-diacylglycerol. The latter compound in combination with intracellular calcium then causes the translocation and activation of PKC. Activated PKC may phosphorylate secondary effectors, which would be responsible for the protective effects of ischaemic preconditioning (Speechly-Dick *et al.*, 1994; Brew *et al.*, 1995).

It may be argued that the protective effect of BK could be based on an increase in coronary flow, which would increase glucose uptake and washing of deleterious catabolic wastes. However, an increase in coronary flow is certainly not the only contributor of these beneficial effects. Previous studies have shown that BK at a low concentration, without any effect on coronary flow, still prevented the deleterious effect of ischaemia-reperfusion (Brew *et al*., 1995; Linz *et al*., 1995). In addition, our study demonstrated protection to the endothelium without a discernible increase in coronary flow.

In conclusion, these data suggest that ischaemic preconditioning affords protection to the endothelial function against subsequent ischaemic insult in the intact coronary circulation of the rat. The reduced protective effect of ischaemic preconditioning in presence of LLDBK suggests that this protection may be mediated partially by B_1 receptor activation. The lack of an inhibitory action of Hoe 140 on ischaemic preconditioning suggests that B_2 receptors do not play a role in the endogenous protection of the endothelial function. Exogenous perfusion of BK or DBK can afford protection to endothelial function against the deleterious effect of ischaemia-reperfusion.

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