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Participation of prostaglandin E_2 in the endothelial protective effect of ischaemic preconditioning in isolated rat heart

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Abstract

Objective: To assess whether the protective effect of ischaemic preconditioning (IPC) on endothelial function in coronary arteries of the rat involves prostaglandins. **Methods:** Isolated rat hearts perfused under constant flow conditions were exposed to 30 min of partial ischaemia (flow-rate 1 ml/min) followed by 20 min of reperfusion, after which coronaries were precontracted with U-46619 0.1 μM, and the coronary response to the endothelium-dependent vasodilator, serotonin (5-HT, 10 μM), was compared to that of the endothelium-independent vasodilator, sodium nitroprusside (SNP, 3 μM). Prostaglandin production was blocked with a perfusion of indomethacin 10 μM started 15 min before IPC or a corresponding sham period and stopped just before the 20-min reperfusion period. **Results:** In untreated hearts, ischaemia diminished selectively 5-HT-induced vasodilatation, compared to sham hearts. The vasodilatation by SNP was unaffected after ischaemia and reperfusion. IPC (5 min of zero-flow ischaemia followed by 10 min reperfusion before the 30-min partial ischaemia) preserved the vasodilatation produced by 5-HT. Enzymeimmunoassays showed an increased production of PGE_2 in the IPC group. Treatment of hearts with indomethacin blocked the protective effect of IPC on the vasodilatation produced by 5-HT and decreased the production of PGE_2. A 5-min perfusion with 3 nM PGE_2 started 15 min before the partial ischaemia, protected the endothelium. This was blocked by 1 μM chelerythrine, but not by 0.3 μM glibenclamide. **Conclusions:** These results suggest that IPC affords protection to endothelial function in coronary arteries of the rat partially via the release of PGE_2. Under our experimental conditions, the protective effect of PGE_2 is mediated by PKC.

Keywords: Endothelial factors; Ischemia; Preconditioning; Prostaglandins; Reperfusion

1. Introduction

Single or repetitive short periods of ischaemia followed by intermittent reperfusions, render the heart more resistant to a subsequent longer ischaemic period. This phenomenon described for the first time by Murry et al. [1] limits infarct size [1,2], reduces the risk of ischaemia–reperfusion arrhythmias [3,4], improves recovery of ventricular function [2], reduces catabolite accumulation, and slows ischaemic metabolism [5,6]. This cardioprotective effect called ischaemic preconditionnig (IPC) has been observed in different species, including rats [7,8], rabbits [2], dogs [1], pigs [9], and humans [10].

Some studies have demonstrated that ischaemia–reperfusion attenuated endothelial function in large coronary vessels [11,12] and in coronary microvessels [13]. In addition, it has been shown that the beneficial effect of IPC is not limited to the cardiomyocytes, but can be observed in endothelial cells in various experimental models including dog resistance coronary arteries in vivo [13], and coronary circulation of the rat in vitro [14,15]. Adenosine [15–17], ATP sensitive potassium channels (K_{ATP} channels) [15,18,19], and protein kinase C (PKC) activation [20,21] have all been implicated as mechanisms of the protection afforded by IPC.

On the other hand, ischaemia and hypoxia are known to be potent stimuli of prostaglandin release into the coronary circulation [22–26]. Furthermore, a number of reports have suggested that cyclooxygenase (COX) products may be involved in the protective effects of myocardial precondi-

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tioning. With respect to COX-derived metabolites, Vegh et al. [27] reported that the antiarrhythmic effect of IPC in the canine heart was abolished by the COX inhibitor, meclofenamate, although the effects of this drug on either infarct size or ventricular haemodynamics were not determined. Another group reported that aspirin abolished the effect of IPC against reperfusion tachyarrhythmias in isolated Langendorff perfused rat hearts [28]. In contrast, some groups failed to observe any effect of aspirin in the rat heart subjected to coronary artery occlusion followed by reperfusion, either in terms of infarct size [29] or the incidence of arrhythmias [29,30].

In addition, some studies have demonstrated that exogenous prostaglandin perfusion preserves myocardial metabolism [31] and decreases infarct size [32]. Moreover, defibrotide, an agent capable of increasing prostacyclin production by endothelial cells significantly protected rat heart, kidney and liver from ischaemia and postischaemic reperfusion injuries [33]. But, on the other hand, experimental studies with exogenous prostaglandins have produced conflicting results with respect to their influence on ischaemia–reperfusion induced ventricular fibrillation [34–36].

However, for the moment, little is known about the role played by endogenous prostaglandins in the endothelial protective effect of IPC. Therefore, the first aim of the present study was to evaluate whether IPC affords protection against ischaemic injury to the endothelium of coronary vessels in isolated rat hearts via the prostaglandin pathway. The second aim was to verify whether exogenous prostaglandin perfusion could mimic the beneficial effects of IPC against ischaemic injury in these hearts.

2. Methods

2.1. 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 narcoticised with CO_2 until a complete loss of consciousness and rapidly decapitated. Hearts were rapidly excised and immersed in ice-cold heparinised Krebs–Henseleit buffer (10 I.U. ml^{-1}). They were immediately mounted on the experimental Langendorff set-up 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 stabilisation 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 all the experiments with an in-line ultrasonic flow probe and meter (Transonic Systems 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, 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) were 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. The typical systolic pressure values measured with this setup were around 80–85 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).

2.2. Experimental protocols

2.2.1. Ischaemic preconditioning protocol

The animals were assigned to one of three different experimental protocols (Fig. 1, protocols 1–3). The hearts in all groups were subjected to a 20-min stabilisation period. Drugs or vehicle infusion was then started, followed by an additional 15-min perfusion period. The ischaemic groups (protocol 2, Fig. 1) were subjected to a 15-min sham period, followed by 30 min of partial ischaemia (flow-rate 1 ml/min) prior to a 20-min reperfusion period. In the preconditioned groups (protocol 3, Fig. 1), 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 (protocol 1, Fig. 1) 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, an agonist of thromboxane A_2 receptors [37], administered throughout the end of the experiment. 15 min after the beginning of U-46619 infusion, the endothelial function was evaluated by the vasodilatation produced by 10 μM serotonin (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

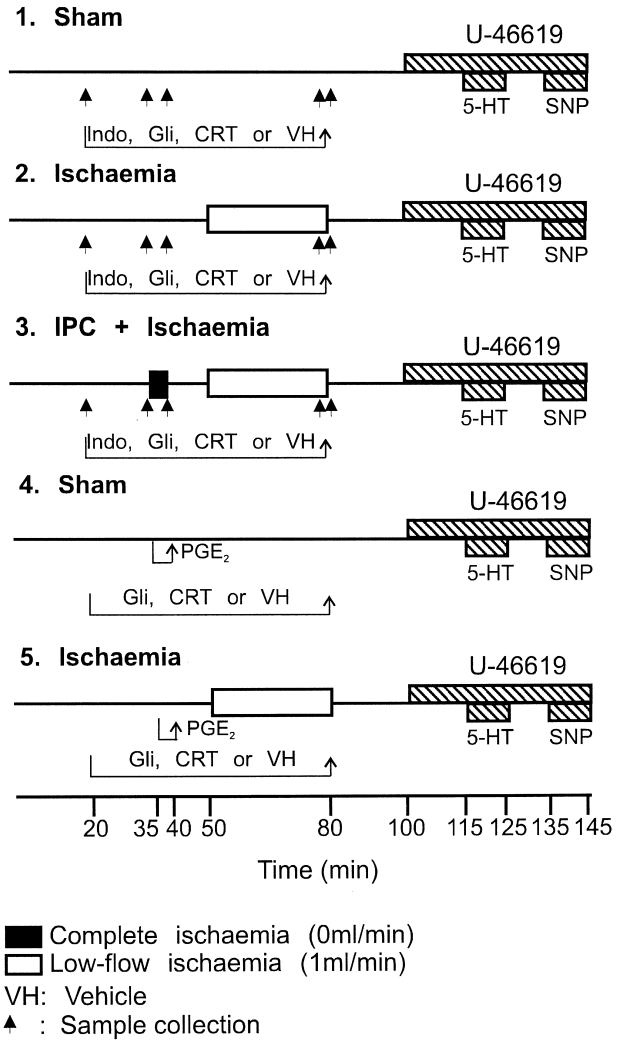

Fig. 1. Diagrams showing the different experimental protocols. Each experiment started with a 20-min stabilisation period. Hearts in the ischaemia protocol (nos. 2 and 5) underwent 30 min of low-flow (1 ml min^{-1}) ischaemia and 20 min of reperfusion, after an additional 30-min stabilisation period. 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 [indomethacin 10 μM (nos. 1–3), glibenclamide 0.3 μM (nos. 1–5), chelerythrine 1 μM (nos. 1–5) or their respective vehicles (all protocols)] was started after the 20-min stabilisation period, lasted throughout the 30-min low-flow ischaemia or a corresponding period, and was stopped upon reperfusion. The 5-min perfusion of prostaglandin E_2 3 nM (nos. 4 and 5) was started 15 min before the 30-min low-flow ischaemia or a corresponding period. For 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 SNP (3 μM, 10 min) infusions. The abscissa represents the time in min.

washout period of 10 min was allowed between each infusion. Vasodilatation was evaluated by computing percent 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 preconditioning hearts were treated with either 10 μM indomethacin, 0.3 μM glibenclamide, 1 μM chelerythrine or their respective vehicles starting after the 20-min stabilisation period, and lasting throughout the 30-min partial ischaemic period (protocols 1–3, Fig. 1). Drug infusion was stopped upon reperfusion.

2.2.2. Exogenous PGE_2 experimental protocol

In additional experimental series, an exogenous prostaglandin E_2 (PGE_2) perfusion was performed instead of ischaemic preconditioning. The animals were assigned to one of two different experimental protocols (protocols 4 and 5, Fig. 1). The hearts in all groups were subjected to a 20-min stabilisation period. Drugs or vehicle infusion was then started, followed by an additional 15-min perfusion period. The ischaemic groups (protocol 5, Fig. 1) were subjected to 30 min of partial ischaemia (flow-rate 1 ml/min) prior to a 20-min reperfusion period. In these groups, hearts were pretreated with either 0.3 μM glibenclamide, 1 μM chelerythrine or vehicles starting after the 20-min stabilisation period, in order to expose the hearts to 15 min antagonist perfusion before the exposure to PGE_2. The antagonist perfusion lasted throughout the 30-min low-flow ischaemic period, and was stopped upon reperfusion. PGE_2 was perfused for 5 min, 15 min before the 30-min low-flow ischaemia. The sham groups (protocol 4, Fig. 1) 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 serotonin (5-HT), whereas coronary smooth muscle function was evaluated with 3 μM sodium nitroprusside (SNP).

2.2.3. 6-Keto $PGF_{1\alpha}$ and PGE_2 determination in coronary effluent

Coronary effluent samples were collected five times for each protocol (after the 20-min stabilisation period, just before zero-flow ischaemic period, 5 min after (upon reperfusion), 29 min after the beginning of the low-flow ischaemic period, and 30 s after the beginning of reperfusion (Fig. 1). Samples for measurement of prostacyclin (PGI_2) release were kept at room temperature for 15 min to ensure a complete hydrolysis of PGI_2 into its stable metabolite, 6-keto $PGF_{1\alpha}$. All the samples were then stored at −20°C until analysis. The concentration of 6-keto $PGF_{1\alpha}$ and PGE_2 in coronary effluent samples were evaluated by enzymeimmunoassays (EIA) (Biotrak, Amersham, Life Science, Buckinghamshire, UK).

2.3. Statistical analysis

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

2.4. Drugs

Drugs were obtained from Sigma-Aldrich (Mississauga, Canada). Indomethacin (1 mM), was prepared in 600 μl 100% dimethylsulphoxide (DMSO) and 30 ml of Krebs–Heinseleit buffer. Chelerythrine (5 mM) was dissolved in bidistilled water. A stock solution of glibenclamide (10 mM) was prepared in 100% DMSO. Stock solution of PGE_2 (2.84 mM) was prepared in 100% ethanol. All these stock solutions were diluted in Krebs–Henseleit buffer to obtain the desired final concentrations. 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%) and DMSO (0.02%), 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 directly in Krebs–Henseleit buffer.

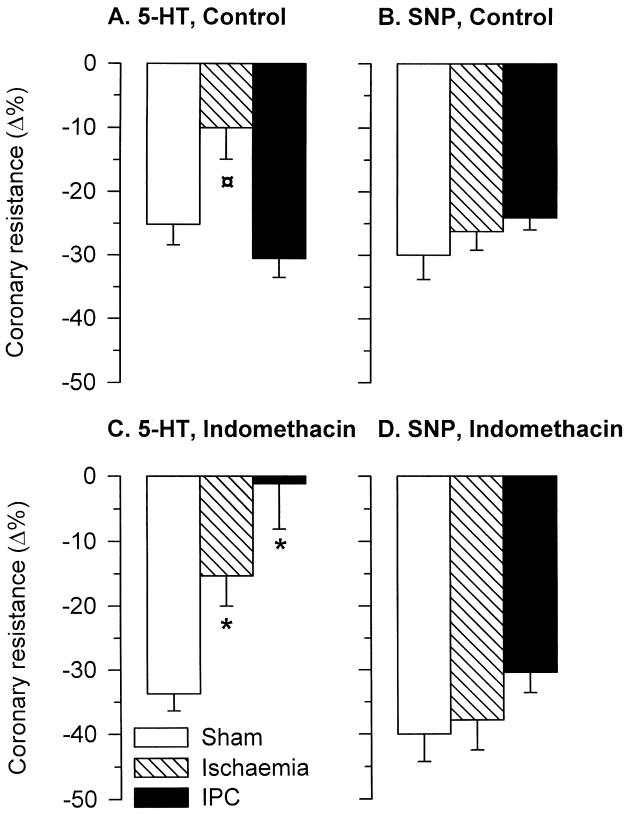

Fig. 2. Change in coronary resistance (Δ%) induced by 10 μM serotonin (5-HT, A and C) and 3 μM sodium nitroprusside (SNP, B and D) in untreated hearts (A and B), and in 10 μM indomethacin pretreated hearts (C and D). Open, hatched, and closed columns represent sham, ischaemic, and ischaemic preconditioning protocols, with 8, 8, and 9 untreated and 7, 8, and 4 indomethacin pretreated hearts, respectively. ¤ $P<0.05$, compared with sham and ischaemic preconditioning. *$P<0.05$, compared with sham (one-way ANOVA with Scheffe post-hoc test).

3. Results

3.1. Ischaemic preconditioning groups

3.1.1. Vascular function

3.1.1.1. Untreated groups. Coronary resistance measured just before 0.1 μM U-46619 perfusion ($n=25$) was 5.94±0.29 mmHg min ml^{-1}, for a coronary flow-rate of 6.70±0.20 ml min^{-1} g^{-1} (mean heart weight of 1.90±0.05 g). Infusion of U-46619 (0.1 μM, $n=25$) induced a significant ($P<0.05$) vasoconstriction in all groups of hearts (sham, ischaemia, and IPC). Perfusion of 10 μM 5-HT produced a decrease in coronary resistance of −25.2±3.3% in sham heart ($n=8$). 30 min of low-flow ischaemia ($n=8$) significantly diminished the 5-HT-induced vasodilatation by more than half (Fig. 2A). One period of IPC ($n=9$) 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 (Fig. 2A). Endothelium-independent vasodilatation to 3 μM SNP was not affected by ischaemia and was found to be comparable in the three groups of hearts (sham, ischaemia and IPC, Fig. 2B).

3.1.1.2. Indomethacin-treated groups. Inhibition of COX-1 with indomethacin (10 μM) produced no significant change in coronary resistance when measured just before 0.1 μM U-46619 perfusion (6.50±0.50, $n=19$ vs. 5.94±0.29 mmHg min ml^{-1}, $n=25$ in untreated hearts, $P>0.05$). The perfusion rate was 5.53±0.30 ml min^{-1} g^{-1} (mean heart weight of 2.23±0.07 g). Infusion of U-46619 (0.1 μM, $n=19$) induced a significant ($P<0.05$) vasoconstriction in all indomethacin-treated groups (coronary resistance of 10.27±0.67 mmHg min ml^{-1}, 10.75±0.85 mmHg min ml^{-1}, and 10.87±1.38 mmHg min ml^{-1} in sham ($n=7$), ischaemic ($n=8$) and IPC ($n=4$) groups, respectively). Vasodilatation produced by 10 μM 5-HT (−33.7±2.7% in sham hearts, $n=7$) was almost totally abolished in the ischaemic group ($n=8$) (Fig. 2C). IPC in indomethacin-treated hearts ($n=4$) was unable to prevent the deleterious effect of ischaemia on 5-HT-induced

vasodilatation (Fig. 2C). Vasodilatation to 3 μM SNP was comparable in the three indomethacin-treated groups (sham, ischaemic and IPC, Fig. 2D).

3.1.1.3. Glibenclamide-treated groups. Blockade of K_{ATP} channels with glibenclamide (0.3 μM) ($n=24$) was accompanied by a significant increase in coronary resistance when measured just before 0.1 μM U-46619 perfusion [glibenclamide-treated (8.89±0.49 mmHg min ml^{-1}) vs. untreated hearts (5.94±0.29 mmHg min ml^{-1}, $P<0.05$)]. The perfusion rate was 5.30±0.13 ml min^{-1} g^{-1} (mean heart weight of 1.94±0.04 g). Infusion of U-46619 (0.1 μM, $n=24$) induced a significant ($P<0.05$) vasoconstriction in glibenclamide-treated group (13.62±0.75 mmHg min ml^{-1}). Vasodilatation produced by 10 μM 5-HT (−15.6±1.6% in sham hearts, $n=8$) was totally abolished in the ischaemic group (Fig. 3A). IPC in glibenclamide-treated hearts failed to prevent the deleterious effect of ischaemia on 5-HT-induced vasodilatation (Fig. 3A). Vasodilatation to 3 μM SNP was comparable in the three glibenclamide-treated groups (sham, ischaemic and IPC, Fig. 3B).

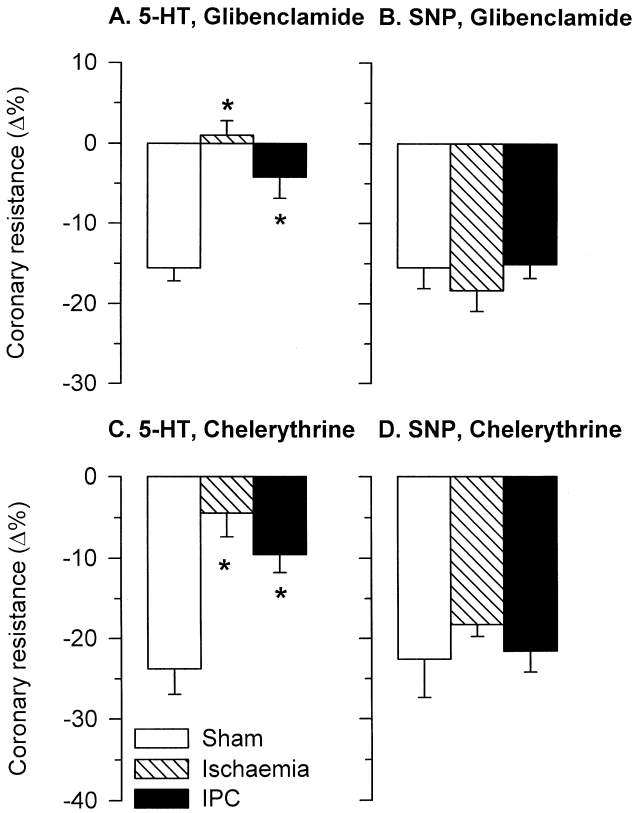

Fig. 3. Change in coronary resistance (Δ%) induced by 10 μM serotonin (5-HT, A and C) and 3 μM sodium nitroprusside (SNP, B and D) in 0.3 μM glibenclamide pretreated hearts (A and B), and in 1 μM chelerythrine pretreated hearts (C and D). Open, hatched, and closed columns represent sham, ischaemia, and ischaemic preconditioning protocols, with 8, 8, and 8 glibenclamide, and 7, 7, and 6 chelerythrine pretreated hearts, respectively. *$P<0.05$, compared with sham (one-way ANOVA with Scheffe post-hoc test).

3.1.1.4. Chelerythrine-treated groups. Inhibition of protein kinase C with chelerythrine (1 μM) produced no significant change in coronary resistance when measured just before 0.1 μM U-46619 perfusion (chelerythrine-treated (6.87±1.48 mmHg min ml^{-1}) vs. untreated hearts (5.94±0.29 mmHg min ml^{-1}), $P>0.05$). The perfusion rate was 5.36±0.24 ml min^{-1} g^{-1} (mean heart weight of 2.25±0.06 g). Infusion of U-46619 (0.1 μM, $n=20$) induced a significant ($P<0.05$) increase of the coronary resistance (12.45±0.45 mmHg min ml^{-1}). Vasodilatation produced by 10 μM 5-HT (−23.8±3.2% in sham hearts, $n=7$) was practically abolished in the ischaemic group (Fig. 3C). IPC in chelerythrine-treated hearts was unable to prevent the deleterious effect of ischaemia on 5-HT-induced vasodilatation (Fig. 3C). Vasodilatation to 3 μM SNP was comparable in the three chelerythrine-treated groups (sham ($n=7$), ischaemic ($n=7$), and IPC ($n=6$), Fig. 3D).

3.1.2. Myocardial function

The inotropic characteristics of indomethacin, glibenclamide and chelerythrine-pretreated hearts were comparable to those of untreated hearts: dP/dt_{max} values measured before the 30-min low-flow ischaemia were 1980±449 ($n=25$), 1613±106 ($n=19$), 1775±119 ($n=24$), and 1639±137 mmHg s^{-1} ($n=20$) for untreated, indomethacin, glibenclamide, and chelerythrine-pretreated hearts, respectively. Low-flow ischaemia was accompanied by a severe reduction in dP/dt_{max} in all groups, which was followed by a complete recovery within 20 min of reperfusion (data not shown). Left ventricular systolic pressure followed the same pattern as dP/dt_{max}, with a severe reduction during ischaemia and a complete recovery upon reperfusion, whereas diastolic pressure was not significantly affected (data not shown). All parameters of myocardial function (systolic and diastolic pressure, dP/dt_{max}, and dP/dt_{min}) at the end of the reperfusion period were comparable in all groups studied.

3.1.3. Prostaglandin measurements in coronary effluent

3.1.3.1. 6-Keto $PGF_{1\alpha}$. Before indomethacin treatment (T20), 6-keto $PGF_{1\alpha}$ measured in the coronary effluent was 664±56 pM, $n=16$ (Fig. 4). Treatment with indomethacin produced a significant decrease in 6-keto $PGF_{1\alpha}$ levels just before the 5-min zero-flow ischaemic period (T35) and after this period (T40) ($P<0.05$). At the end of the low-flow ischaemia (T79), levels of 6-keto $PGF_{1\alpha}$ were significantly increased in the ischaemic un-

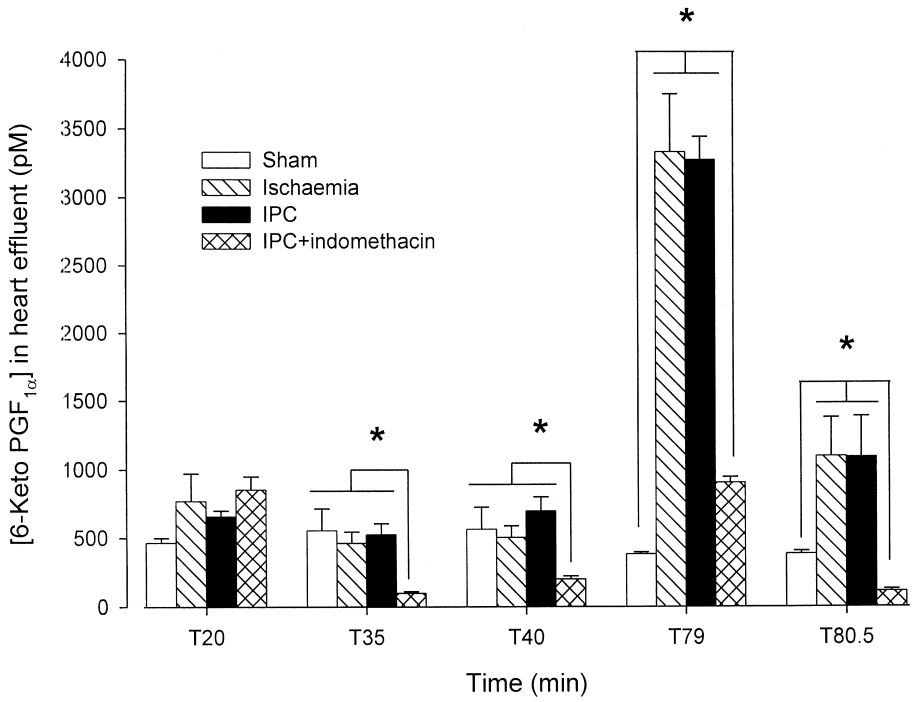

Fig. 4. Coronary effluent concentration of 6-keto $PGF_{1\alpha}$ (pM) after stabilisation period (T20), at the beginning of the 5-min zero-flow ischaemia (T35), 10 min before low-flow ischaemia (T40), at the end of the low-flow ischaemia (T79) and 30 s after the beginning of reperfusion (T80.5). *$P<0.05$ compared with the indicated group(s), two-way ANOVA with Scheffe post-hoc test.

treated group ($n=3$) and in the IPC untreated group ($n=4$) vs. the sham untreated group ($n=3$) ($P<0.05$). This increase in 6-keto $PGF_{1\alpha}$ levels was significantly blocked by indomethacin ($n=6$) at time T79 and T80.5 ($P<0.05$).

3.1.3.2. PGE_2. Before indomethacin treatment (T20), PGE_2 in the coronary effluent amounted to 380±31 pM, $n=21$ (Fig. 5). Treatment with indomethacin produced a significant decrease in PGE_2 levels just before the 5 min zero-flow ischaemic period (T35) and after this period (T40) ($P<0.05$). At T40, IPC produced a significant increase in PGE_2 levels ($n=4$). At the end of the low-flow ischaemia (T79), levels of PGE_2 were significantly increased in the ischaemic untreated group ($n=3$) and in the IPC untreated group ($n=4$) vs. the sham untreated group ($n=3$) ($P<0.05$). At T79, the increase in IPC group ($n=4$) was significantly higher from that of the ischaemic group ($n=3$) ($P<0.05$). These increases in PGE_2 levels were significantly blocked by indomethacin ($n=6$) at time T79 and T80.5 ($P<0.05$).

3.2. Exogenous prostaglandin groups

3.2.1. Vascular function

Perfusion with PGE_2 (3 nM) produced a slight but significant decrease in coronary resistance when measured five min after the beginning of its perfusion (7.9±4.8% decrease of coronary resistance, $n=12$). Coronary resistance measured just before 0.1 μM U-46619 perfusion ($n=12$) was 6.83±0.67 mmHg min ml^{-1} and the perfusion rate in PGE_2-treated hearts was 4.86±0.19 ml min^{-1} g^{-1} (mean heart weight of 2.12±0.06 g). Infusion of U-46619 (0.1 μM, $n=12$) induced a significant ($P<0.05$) vasoconstriction in PGE_2-treated hearts (14.07±0.59 mmHg min ml^{-1}). 30 min of low-flow ischaemia significantly diminished the 5-HT-induced vasodilatation by more than half in untreated hearts (Fig. 2A). Treatment with PGE_2, starting 15 min before ischaemia and lasting only 5 min, preserved the vasodilatation produced by 10 μM 5-HT in ischaemic hearts (Fig. 6A). Vasodilatation to 3 μM SNP was comparable in all PGE_2-treated hearts (Fig. 6B). Treatment with 5 min PGE_2 perfusion in glibenclamide-pretreated hearts prevented the deleterious effect of ischaemia on 5-HT-induced vasodilatation (Fig. 6C). In contrast, treatment with PGE_2 in chelerythrine-pretreated hearts was unable to prevent the deleterious effect of ischaemia on 5-HT-induced vasodilatation (Fig. 6E). Vasodilatation to 3 μM SNP was comparable in all groups, regardless of the treatment (Fig. 6 B,D,F)

3.2.2. Myocardial function

In all groups, pretreatment with exogenous PGE_2 perfusion for 5 min, 30-min before low-flow ischaemia, had no

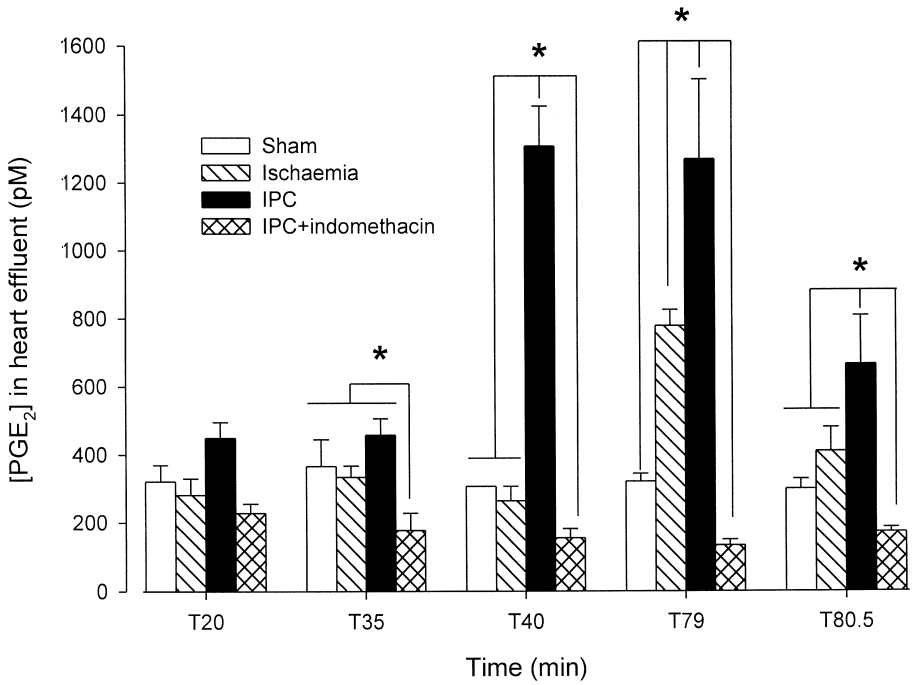

Fig. 5. Coronary effluent concentration of PGE_2 (pM) after stabilisation period (T20), at the beginning of the 5-min zero-flow ischaemia (T35), 10 min before low-flow ischaemia (T40), at the end of the low-flow ischaemia (T79) and 30 s after the beginning of reperfusion (T80.5). *$P<0.05$ compared with the indicated group(s) (two-way ANOVA with Scheffe post-hoc test).

effect on ischaemic or post-ischaemic recovery of dP/dt_{max}.

4. Discussion

In the present study, we have assessed the contribution of prostaglandins in the protective effect of IPC on endothelial function in the rat isolated heart. The major findings are (1) that IPC with a single short period of ischaemia prevents endothelial dysfunction produced by ischaemia–reperfusion in rat hearts partially via the release of PGE_2, (2) this protection can be blocked by a pretreatment with indomethacin, and (3) 5-min PGE_2 perfusion starting 15 min before ischaemia mimics the beneficial effect of IPC on endothelial function in rat coronary arteries.

The rat isolated heart has, like any experimental model, its advantages and limitations. The hearts were not perfused during the few seconds it took to mount them on the perfusion setup. This could theoretically produced an IPC per se. However, the absence of perfusion during the preparation was not severe enough to protect the endothelial function under the present experimental conditions. In addition, the isolated hearts were not perfused with blood, but with an oxygenated buffer. Although this can limit the comparison of our results to the one obtained in vivo, this model provides a valuable tool to study the local protective mechanisms without any interference with blood-borne elements.

4.1. Effect of ischaemic 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 [38] vasodilatation of coronary arteries to 5-HT was drastically decreased after ischaemia–reperfusion insult, whereas the same vessels retained the ability to dilate to SNP, an endothelium-independent vasodilator. We have reported earlier [15] that IPC could prevent the reduction in the vasodilatation to 5-HT after ischaemia–reperfusion, suggesting that ischaemic preconditioning could protect endothelial function in coronary arteries. Such a protection was observed in the present study. A protective effect of IPC was also observed with canine epicardial coronary arteries [13], and in rat isolated left coronary arteries in vitro [14].

4.2. Role of prostaglandins in ischaemic preconditioning

Prostaglandins have been reported to be endogenous mediators of the protection afforded by IPC [27,28]. Some investigators have reported that a COX inhibitor, meclofenamate, prevented the protective effect of IPC on arrhythmias in dogs in vivo [27]. Another group demonstrated that aspirin could abolish the antiarrhythmic effect of IPC against reperfusion tachyarrhythmias in isolated rat hearts [28]. However, to our best knowledge, little is known about the role played by endogenous prostaglandins

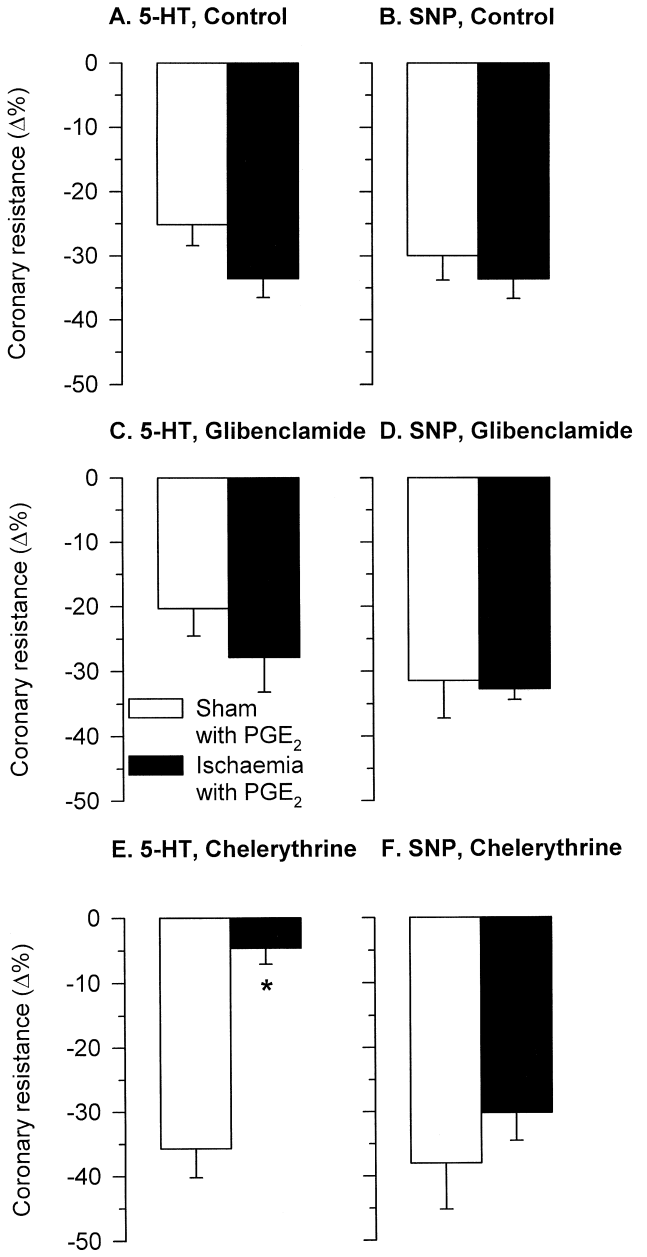

Fig. 6. Change in coronary resistance (Δ%) induced by 10 μM serotonin (5-HT, A, C and E) and 3 μM sodium nitroprusside (SNP, B, D and F) in untreated hearts (A and B), in glibenclamide pretreated hearts (C and D), and in chelerythrine pretreated hearts (E and F). Open and closed columns represent sham with 3nM PGE_2 and ischaemia with PGE_2 protocols, with 6 and 6 untreated, 8 and 8 glibenclamide, and 8 and 8 chelerythrine pretreated hearts, respectively. *$P<0.05$, compared with sham (two-way ANOVA).

in the endothelial protective effect of IPC. Therefore, we tested, using indomethacin, whether prostaglandins were involved in the protection afforded by IPC to the endothelium. In the indomethacin-treated and preconditioned group, the vasodilatation to 5-HT was almost totally abolished whereas the vasodilatation to SNP was not significantly different from that observed in the sham group. These data suggest that endogenously produced cyclooxygenase metabolites play a role in the endothelial protection afforded by IPC.

In the untreated group, low-flow ischaemia was accompanied by an important increase in 6-keto $PGF_{1\alpha}$ and PGE_2 levels measured in the coronary effluent. However, only PGE_2 levels increased during IPC and its accumulation at the end of the low-flow ischaemia in the preconditioned group was significantly higher than that observed in the non-preconditioned ischaemic group. Pretreatment with indomethacin 15 min before and during ischaemia, blocked these increases in prostaglandin levels and abolished the protective effect of IPC. Therefore, these data suggest that increased PGE_2 concentration during IPC and low-flow ischaemia is one of the mechanisms involved in the endothelial protection induced by IPC. This however contrasts with the finding of Arad et al. [28] who reported that IPC was associated with an increased release of prostacyclin prior to reperfusion in the isolated hearts. The reason for this discrepancy remains unclear.

A few years ago, we reported that PGE_2 could activate ATP-sensitive potassium (K_{ATP}) channels [39]. In the myocardium, activation of K_{ATP} channels has been linked to cardioprotection, possibly through a reduction in intracellular Ca^{2+} levels [19], prevention of mitochondrial Ca^{2+} overload or preservation of the myocardial energy status [40,41]. In endothelial cells, activation of K_{ATP} channels produces hyperpolarisation, which increases the electrochemical gradient for Ca^{2+} entry [42,43], resulting in an enhanced nitric oxide release [44].

PGE_2 can also act on EP_2 receptors, stimulating the production of cyclic adenosine 3′,5′-monophosphate and inducing vasodilatation [45]. Alternatively, EP_1 receptors activate phospholipase C to release inositol 1,4,5-trisphosphate and 1,2-diacylglycerol [45]. The latter compound in combination with intracellular calcium then causes the translocation and activation of protein kinase C (PKC) [46]. Activated PKC may phosphorylate secondary effectors, which would be responsible for the protective effects of PGE_2. PKC activation have been implicated in the mechanisms of the protection afforded to the myocardium by IPC [20,21].

Therefore, we tested, using glibenclamide, a K_{ATP} channel blocker, and chelerythrine, a PKC inhibitor, whether these were involved in the protection afforded by IPC, as well as exogenous PGE_2, to the endothelium under our experimental conditions.

In the glibenclamide-treated groups, the protective effect of IPC on endothelial function was diminished by more than half, as reflected by the reduction of endothelium-dependent vasodilatation to 5-HT in the preconditioned group, compared to the glibenclamide-treated sham group. Likewise, in the chelerythrine-treated groups, the protective effect of IPC on endothelial function was halved, as reflected by the reduction of endothelium-dependent vasodilatation to 5-HT in the preconditioning group, compared to the chelerythrine-treated sham group. The vasodilatation

to SNP was not affected by these treatments. These data suggest that both K_{ATP} channels and PKC activation are involved in the protection afforded by IPC against endothelial dysfunction observed with ischaemia–reperfusion. Interestingly, ischaemia appeared to be more deleterious for the endothelium in the presence of glibenclamide and, to a lesser extent, chelerythrine. This suggests that K_{ATP} channels and PKC play a protective role even in the absence of IPC, the latter having an enhancing effect on these protective mechanisms.

4.3. Protective effect of exogenous PGE_2

To confirm the contribution of PGE_2 in the endothelial protection afforded by IPC, the effect of exogenous perfusion with a low concentration of PGE_2 on the endothelial function following ischaemia–reperfusion was studied. PGE_2 perfused for 5 min, 15 min before ischaemia prevented the ischaemia-induced reduction in the vasodilatation to 5-HT. Pretreatment with chelerythrine, but not with glibenclamide, perfused 15 min before and during low-flow ischemia, abolished the protective effect of PGE_2 on 5-HT vasodilatation. Thus, these data suggest that PGE_2 can mimic the protective effect of IPC on the endothelial function via the activation of PKC. Under our experimental conditions, K_{ATP} channels seem not to be implicated in this protection. This implies that other mechanisms or autacoids, like adenosine, are responsible for K_{ATP} channel activation by IPC.

In conclusion, these data suggest that IPC affords protection to the endothelial function against subsequent ischaemic injury in the intact coronary circulation of the rat. The reduced protective effect of IPC in presence of indomethacin and the increase of PGE_2 levels during IPC suggest that this protection may be mediated partially via the production of PGE_2. Exogenous perfusion of PGE_2 can afford protection to the endothelial function against the deleterious effect of ischaemia–reperfusion via an activation of protein kinase C.

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