

Protection afforded by preconditioning to the diabetic heart against ischaemic injury

Jean-François Bouchard, Daniel Lamontagne *

Faculty of Pharmacy, University of Montreal, C.P. 6128, Succursale Centre-ville, Montreal, Québec, Canada, H3C 3J7

Received 9 April 1997; accepted 20 August 1997

Abstract

Objective: The aim of this study was to assess whether the cardioprotective effect of ischaemic preconditioning (IPC) on endothelial function in coronary arteries and myocardial function is affected in the streptozotocin-induced diabetic rat heart. **Methods:** Isolated hearts, perfused under constant flow conditions, were exposed to 30 min of partial ischaemia (flow rate 1 ml min^{-1}) followed by 20 min of reperfusion. **Results:** In the diabetic group (without ischaemia or IPC), infusion of $10 \text{ } \mu\text{M}$ serotonin (5-HT), an endothelium-dependent, and $3 \text{ } \mu\text{M}$ sodium nitroprusside (SNP), an endothelium-independent vasodilator, in the coronary bed precontracted with $0.1 \text{ } \mu\text{M}$ U-46619 induced a marked vasodilation. Ischaemia, either without or with preconditioning with a single 5 min ischaemia and 10 min reperfusion (IPC1) before ischaemia, was accompanied by a reduced 5-HT-induced vasodilation in diabetic hearts. In contrast, IPC1 preserved the response to 5-HT in non-diabetic hearts. A more extensive IPC with 3 periods of 5 min ischaemia followed by 5 min reperfusion (IPC3) preserved the vasodilation produced by 5-HT in both diabetic and non-diabetic hearts. IPC3 increased the recovery of dP/dt_{max} and dP/dt_{min} during the 30 min ischaemic period and during reperfusion in all hearts. In contrast, IPC1 had no effect on myocardial recovery in either groups. Adenosine pre-treatment started 30 min before ischaemia mimicked IPC3, preserving the vasodilation to 5-HT and improving myocardium recovery in both groups. When adenosine was started 15 min before ischaemia, vasodilation to 5-HT was preserved in non-diabetic hearts only. **Conclusions:** These results suggest that IPC affords protection to endothelial function in resistance coronary arteries of diabetic hearts. To achieve this protection, a more extensive IPC is needed, which may be related to a longer exposure to adenosine. © 1998 Elsevier Science B.V.

Keywords: Coronary circulation; Diabetes; Endothelium; Heart; Ischaemic preconditioning; Rat

1. Introduction

Single or repetitive short periods of ischaemia followed by intermittent reperfusion, render the heart more resistant to a subsequent longer ischaemic period. This phenomenon, called ischaemic preconditioning (IPC), limits infarct size [1–3], reduces the risk of ischaemia-reperfusion arrhythmias [4–6], improves recovery of ventricular function [7,8], reduces catabolites accumulation, and slows ischaemic metabolism [2]. This cardioprotective effect has been observed in different species, including rats [9,10], rabbits [11], dogs [1], pigs [12], and humans [13].

Some studies have demonstrated that ischaemia-reperfusion attenuated endothelial function in large coronary ves-

els [14,15] and in coronary microvessels [16]. Some groups have demonstrated 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 [16], and coronary arteries of the rat in vitro [17,18]. Adenosine has often been reported to be a mediator of the protection afforded by IPC [19,20]. For example, we have recently demonstrated that blockade of adenosine-receptors with 8-phenyltheophylline can prevent the protection of the endothelial function afforded by IPC in the non-diabetic rat coronary bed [18].

An early study reported that IPC can reduce infarct size in a non-insulin-dependent diabetic rat model in vivo [21]. On the other hand, IPC failed to reduce the incidence of

* Corresponding author. Tel. (+1-514) 343 5909; Fax (+1-514) 343 2102; E-mail: lamontad@ere.umontreal.ca

ventricular arrhythmias and to improve cardiac function in diabetic rat hearts [22,23]. However, to the best of our knowledge, little is known about the effect of IPC on endothelial function in diabetic hearts, and whether exogenous adenosine perfusion can mimic the effects of IPC in this pathological model. Since diabetes has been associated with endothelial [24,25] and myocardial dysfunction [26,27], as well as an altered sensitivity to ischaemic injury [28,29], these unanswered questions are highly relevant.

Therefore, the first aim of the present study was to evaluate whether IPC affords protection against ischaemic injury to the endothelium of coronary vessels and to contractile function in isolated diabetic rat hearts. The second aim was to verify whether exogenous adenosine perfusion can 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 Canadian Council on Animal Care. Male Sprague-Dawley rats weighing 200–225 g were rendered diabetic or treated as vehicle control by injection into the tail vein of streptozotocin (55 mg kg^{-1}) or vehicle (0.1 N citrate buffer, pH 4.5), respectively, under light anaesthesia (methoxyflurane). Animals were allowed free access to food and water at all times. After two months, diabetic rats and age-matched controls were narcotised with CO_2 until complete loss of consciousness and promptly decapitated. The thorax was rapidly opened and the heart excised and immersed in ice-cold heparinised buffer (10 IU ml^{-1}). It was immediately mounted on the experimental setup and perfused within 1 min after decapitation 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 the complete 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, 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). Body weight and blood glucose levels (One Touch II glucometer, Lifescan) were measured at the time of decapitation.

2.2. Experimental protocols

The animals were randomised into twelve groups (Fig. 1). The hearts in all groups were subjected to a 20 min stabilisation period. Ischaemic groups were subjected to a 15 min sham period, followed by 30 min of partial ischaemia (flow rate 1 ml min^{-1}) prior to a 20 min reperfusion period. In the preconditioned groups (IPC), the hearts were exposed to 5 min global ischaemia (zero flow) plus 10 min of reperfusion (IPC1) or 5 min global ischaemia plus 5 min of reperfusion repeated three times (IPC3) before the 30 min ischaemia and 20 min reperfusion periods. The sham groups were not exposed to ischaemia-reperfusion at all, but to a time-matched normal perfusion. After these periods, coronary arteries were precontracted with $0.1 \mu\text{M}$ U-46619 administered throughout the last phase of the experiment. Fifteen min after the beginning of U-46619 infusion, the endothelial function was evaluated with the vasodilation produced by $10 \mu\text{M}$ serotonin (5-HT), whereas coronary smooth muscle function was evaluated using $3 \mu\text{M}$ 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. Vasodilation was quantified 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 vasodilation.

In additional experimental series, the effect of an adenosine perfusion was compared with that of IPC. In these groups, hearts were treated with either $3 \mu\text{M}$ adenosine or vehicle starting after either a 20 min or a 35 min stabilisation period, in order to expose the hearts to either

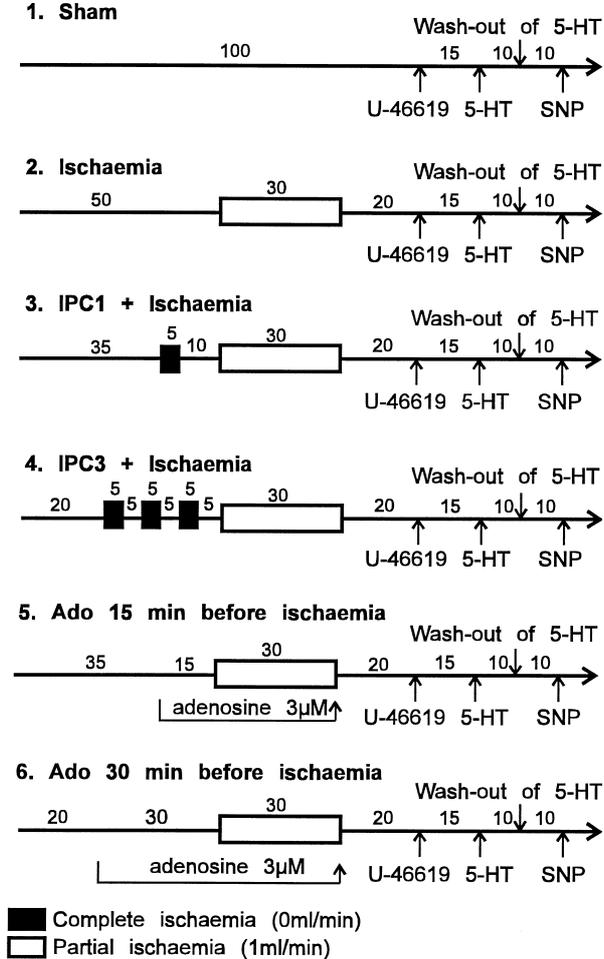


Fig. 1. Diagrams showing the different experimental protocols. Each experiment started with a 20 min stabilisation period. Hearts in the ischaemia protocol (no. 2) underwent 30 min of low-flow (1 ml min⁻¹) ischaemia and 20 min of reperfusion, after an additional 30 min stabilisation period. Hearts in the IPC1 + 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. Hearts in the IPC3 + ischaemia protocol (no. 4) were submitted to 3 cycles of 5 min zero-flow ischaemia followed by 5 min reperfusion, before the 30 min low-flow ischaemia. In the experiments in which adenosine (Ado) perfusion replaced IPC, infusion with 3 μ M adenosine started either 15 min (no. 5) or 30 min (no. 6) before the 30 min low-flow ischaemia and 20 min of reperfusion. Adenosine perfusion lasted throughout the ischaemic period. Hearts in the sham protocol (no. 1) were used as time-matched controls. 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 μ M U-46619. After 15 min, infusion of 5-HT (10 μ M) was started for 10 min. A wash-out period of 10 min was allowed between 5-HT and SNP (3 μ M, 10 min) infusions. The numbers above the bars represent the time in min.

30 or 15 min adenosine perfusion before ischaemia. The adenosine perfusion lasted throughout the ischaemic period.

2.3. Statistical analysis

Values represent the mean \pm 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 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.

2.4. Drugs

All drugs were obtained from Sigma (St. Louis, MA). A 28.5 mM stock solution of U-46619 (9,11-dideoxy-11 α ,9 α -epoxymethano-prostaglandin F_{2 α}) was dissolved in 100% ethanol and diluted with 0.9% NaCl solution to obtain the desired final concentration. Ethanol at the concentration obtained in the final dilution (0.003%), had no effect on any of the hemodynamic variables studied and on the dilator responses to 5-HT and SNP. All the other drugs were dissolved in Krebs-Henseleit buffer.

3. Results

A total of 42 non-diabetic rats and 43 diabetic rats have been used in the present study. Body weight of rats treated with streptozotocin or vehicle two months after injection was 365.3 ± 10.1 and 558.9 ± 13.7 g, respectively, $p < 0.05$. Blood glucose level of these animals was 21.4 ± 0.47 and 4.5 ± 0.12 mmol l⁻¹ respectively, $p < 0.05$.

3.1. IPC groups

3.1.1. Vascular function

Coronary resistance of non-diabetic hearts measured just before 0.1 μ M U-46619 perfusion ($n = 30$) 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). In diabetic hearts ($n = 29$), coronary resistance measured before 0.1 μ M U-46619 perfusion was 4.99 ± 0.32 mmHg min ml⁻¹, for a coronary flow rate of 7.38 ± 0.36 ml min⁻¹ g⁻¹ (mean heart weight of 2.04 ± 0.06 g). Infusion of U-46619 (0.1 μ M, $n = 59$) induced a significant ($p < 0.05$) vasoconstriction in all groups of hearts (sham, ischaemia, IPC1, and IPC3, Table 1). Vasodilation produced by 10 μ M 5-HT in sham hearts from diabetic rats ($-25.2 \pm 4.8\%$) was comparable to that of age-matched control rats ($-25.2 \pm 3.3\%$). Thirty min of partial ischaemia significantly diminished the 5-HT-induced vasodilation by more than half in hearts from non-diabetic and diabetic rats (Fig. 2). One period of IPC in non-diabetic hearts prevented the deleterious effect of ischaemia on endothelium-dependent vasodilation: the vasodilation produced by 5-HT in preconditioned hearts was comparable to that observed in hearts not exposed to ischaemia (Fig. 2). In diabetic hearts, one period of IPC was insufficient to preserve the endothelial function. However, three periods of IPC prevented the deleterious effect of is-

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

	<i>n</i>	Before U-46619	After U-46619
IPC groups			
<i>Non-diabetics</i>			
Sham	8	5.87 \pm 0.35	10.10 \pm 0.82 *
Ischaemia	8	5.96 \pm 0.82	11.53 \pm 0.75 *
IPC1	8	5.94 \pm 0.31	11.53 \pm 0.75 *
IPC3	6	5.25 \pm 0.47	9.50 \pm 0.89 *
<i>Diabetics</i>			
Sham	8	5.35 \pm 0.54	9.34 \pm 1.05 *
Ischaemia	8	5.46 \pm 0.88	10.05 \pm 1.25 *
IPC1	6	5.13 \pm 0.36	8.73 \pm 0.83 *
IPC3	7	3.93 \pm 0.43	7.29 \pm 0.65 *
Adenosine groups			
<i>Non-diabetics</i>			
45 min perfusion	8	5.08 \pm 0.40	8.95 \pm 0.56 *
60 min perfusion	4	5.84 \pm 0.89	9.72 \pm 1.43 *
<i>Diabetics</i>			
45 min perfusion	7	4.51 \pm 0.35	8.63 \pm 0.62 *
60 min perfusion	7	4.78 \pm 0.61	7.56 \pm 0.66 *

Coronary resistance was calculated as perfusion pressure (mmHg)/perfusion flow (ml min^{-1}).

Values are means \pm SEM.

* $p < 0.05$ compared with the corresponding 'before U-46619' value.

chaemia on endothelium-dependent vasodilation in diabetic as well as in non-diabetic hearts (Fig. 2). Endothelium-independent vasodilation to 3 μM SNP was not affected by ischaemia and was found to be comparable in

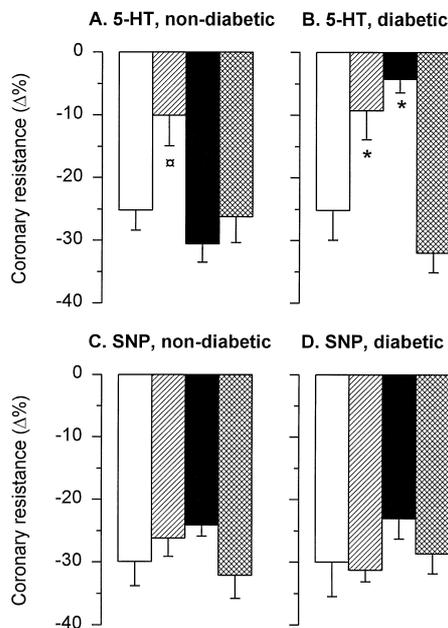


Fig. 2. Change in coronary resistance induced by 10 μM serotonin (5-HT, panels A and B) and 3 μM sodium nitroprusside (SNP, panels C and D) in non-diabetic hearts (panels A and C) and in diabetic hearts (panels B and D). Open, hatched, closed, and crosshatched columns represent sham, ischaemic, IPC1 and IPC3 protocols, with 8, 8, 8, and 6 non-diabetic, and 8, 8, 6, and 7 diabetic hearts, respectively. \square $p < 0.05$, compared with sham, IPC1, and IPC3. * $p < 0.05$, compared with sham and IPC3.

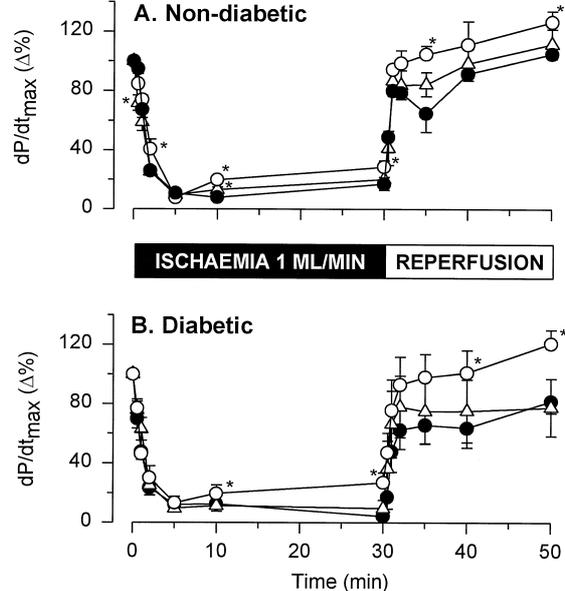


Fig. 3. Change in dP/dt_{max} ($\Delta\%$) observed during 30 min partial ischaemia (1 ml min^{-1}) and 20 min reperfusion in non-diabetic (top panel) and in diabetic hearts (bottom panel). Closed circles, open circles, and open triangles represent ischaemic, IPC3, and IPC1 protocols, with 8, 8, and 6 non-diabetic, and 8, 6, and 7 diabetic hearts, respectively. * $p < 0.05$ compared with ischaemic hearts.

the four groups of hearts (sham, ischaemia, IPC1, and IPC3) from diabetic and non-diabetic rats (Fig. 2).

3.1.2. Myocardial function

The inotropic and lusitropic characteristics of diabetic hearts were comparable to that of non-diabetic hearts: dP/dt_{max} values measured before the 30 min partial ischaemia were 2405 ± 161 and $2307 \pm 117 \text{ mmHg s}^{-1}$, and the dP/dt_{min} values 1695 ± 94 and $1702 \pm 101 \text{ mmHg s}^{-1}$, for diabetic hearts and age-matched controls, respectively. Partial ischaemia was accompanied by a severe reduction in dP/dt_{max} (Fig. 3) in both diabetic and non-diabetic hearts. IPC3 improved dP/dt_{max} (Fig. 3) recovery during ischaemia, particularly during the last 20 min of ischaemia. Reperfusion after partial ischaemia was accompanied by a complete recovery of dP/dt_{max} after 20 min in non-diabetic hearts, whereas diabetic hearts recovered to 80% of the pre-ischaemic level. IPC3 improved the post-ischaemic recovery of dP/dt_{max} (Fig. 3) in diabetic and control rats. In all hearts studied, IPC1 was insufficient to improve ischaemic and post-ischaemic recovery of dP/dt_{max} (Fig. 3). The effect of ischaemia and reperfusion, with or without preconditioning, on dP/dt_{min} in both diabetic and non-diabetic hearts was superimposed with that on dP/dt_{max} (data not shown).

3.2. Adenosine groups

3.2.1. Vascular function

Adenosine perfusion (3 μM) was accompanied by a significant decrease in coronary resistance when measured

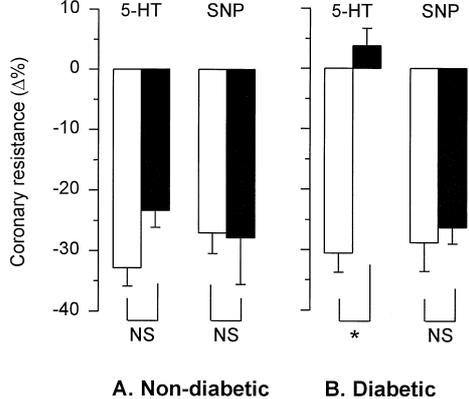


Fig. 4. Change in coronary resistance ($\Delta\%$) induced by $10\ \mu\text{M}$ serotonin (5-HT) and $3\ \mu\text{M}$ sodium nitroprusside (SNP) in non-diabetic (left panel) and in diabetic hearts (right panel). Open and closed columns represent 60 min and 45 min adenosine ($3\ \mu\text{M}$) pre-treatment with 4 and 8 non-diabetic, and 7 diabetic hearts in each pre-treatment, respectively. * $p < 0.05$. NS = non-significant.

just before the 30 min ischaemic period ($-30.5 \pm 2.5\%$ in non-diabetic hearts ($n = 12$) and $-28.1 \pm 3.2\%$ in diabetic hearts, $p < 0.05$, $n = 14$). The perfusion rate in non-diabetic hearts was $6.2 \pm 0.3\ \text{ml min}^{-1}\ \text{g}^{-1}$ (mean heart weight of $2.31 \pm 0.09\ \text{g}$). In diabetic hearts, the perfusion rate was $7.29 \pm 0.39\ \text{ml min}^{-1}\ \text{g}^{-1}$ (mean heart weight of $2.14 \pm 0.09\ \text{g}$). Infusion of U-46619 ($0.1\ \mu\text{M}$, $n = 26$) induced a significant ($p < 0.05$) vasoconstriction in all adenosine-treated hearts (Table 1). Treatment with adenosine, starting either 15 min ($n = 6$) or 30 min ($n = 8$) before ischaemia, preserved the vasodilation produced by

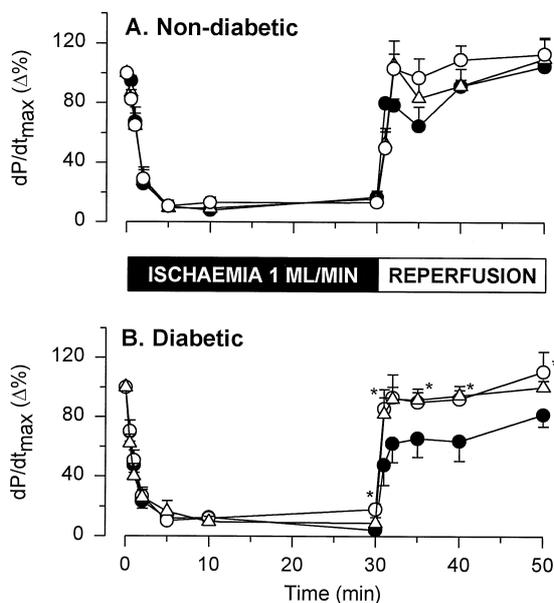


Fig. 5. Change in dP/dt_{max} ($\Delta\%$) observed during 30 min partial ischaemia ($1\ \text{ml min}^{-1}$) and 20 min reperfusion in non-diabetic (top panel) and in diabetic hearts (bottom panel). Closed circles, open circles, and open triangles represent ischaemic, 60 min, and 45 min adenosine pre-treatment protocols for 8, 4, and 8 non-diabetic, and 8, 7, and 7 diabetic hearts, respectively. * $p < 0.05$ compared with ischaemic hearts.

$10\ \mu\text{M}$ 5-HT in non-diabetic hearts (Fig. 4). In contrast, only the 30 min pre-treatment with adenosine ($n = 7$) preserved the vasodilation produced by $10\ \mu\text{M}$ 5-HT in diabetic hearts (Fig. 4). Vasodilation to $3\ \mu\text{M}$ SNP was comparable in all adenosine-treated hearts (Fig. 4).

3.2.2. Myocardial function

The dP/dt_{max} values measured before the 30 min partial ischaemia were 2257 ± 104 and $2000 \pm 64\ \text{mmHg s}^{-1}$, and the dP/dt_{min} values 1574 ± 83 and $1392 \pm 60\ \text{mmHg s}^{-1}$, for diabetic hearts and age-matched controls, respectively. In the control hearts, pre-treatment with adenosine, either 15 or 30 min before ischaemia, had no effect on ischaemic or post-ischaemic recovery of dP/dt_{max} (Fig. 5). In diabetic hearts, the 60 min pre-treatment with adenosine improved dP/dt_{max} recovery during ischaemia, the 45 min pre-treatment having no effect. In contrast, both 45 min and 60 min pre-treatment improved post-ischaemic recovery of dP/dt_{max} (Fig. 5) upon reperfusion in diabetic rats. The effect of ischaemia and reperfusion, with or without adenosine pre-treatment, on dP/dt_{min} in both diabetic and non-diabetic hearts was superimposed with that on dP/dt_{max} (data not shown).

4. Discussion

In the present study, we have evaluated whether IPC can exert a protective effect on myocardial function, and prevent endothelial cell dysfunction induced by ischaemia-reperfusion injury in the coronary circulation of diabetic and non-diabetic rats. The effect of exogenous adenosine perfusion was also evaluated. The major findings of this study are (1) that IPC with a single short period of ischaemia prevents endothelial dysfunction produced by ischaemia-reperfusion in non-diabetic hearts, whereas three periods are necessary in diabetic hearts, (2) IPC with 3 periods of ischaemia can improve the recovery of myocardial function after ischaemia in both groups, (3) adenosine perfusion starting 15 min before ischaemia can mimic the beneficial effect of IPC on endothelial function in non-diabetic coronary arteries, whereas a longer adenosine perfusion (30 min) is obligatory for endothelial protection in diabetic hearts. Finally, the longer adenosine perfusion can also improve the recovery of contractile function after ischaemia in diabetic hearts.

The buffer-perfused, flow-controlled isolated heart was selected for the present study. This model allowed highly reproducible ischaemia and controlled reperfusion without any damage to the vessels by clamping or ligature procedures. However, oxygen transport by a buffer solution is less than that of blood, and a higher flow rate must be used to obtain a perfusion pressure within the physiological range. Since ischaemia-reperfusion can alter the coronary dilatory reserve (Bouchard and Lamontagne, unpublished observation), precontraction of coronary arteries with U-

46619 allowed a more reliable assessment of endothelium-dependent and independent responses. Finally, all groups were time-matched to minimize the effect of time on our preparations.

4.1. Effect of preconditioning on endothelial dysfunction

In the present study, the vasodilation to 5-HT was used as an index of endothelial function. 5-HT has been shown to be an endothelium-dependent vasodilator in several isolated vessel preparations [30] as well as in isolated rat hearts [31]. In isolated hearts, the coronary vasodilation to 5-HT is blocked after treatment with NO-synthase inhibitors [31]. Thus, the vasodilatory response to 5-HT is indicative of the ability of endothelial cells to generate and release NO. The coronary vasodilation to 5-HT was not altered in diabetic rats. This is in contrast with several studies reporting impairment of endothelium-dependent relaxation in aorta from diabetic animals, including rabbits and rats [32,33]. However, other reports have shown no change in endothelium-dependent relaxation in diabetic animals [34,35]. This controversy may be explained by several variables like the animal species, the vascular bed studied, or the duration and severity of the pathology.

Our data show that endothelium-dependent vasodilation of coronary arteries to 5-HT is drastically decreased after ischaemia-reperfusion injury. However the same vessels retained the ability to dilate to SNP, an endothelium-independent vasodilator. This indicates that, under the present conditions, ischaemia-reperfusion altered selectively the functionality of the endothelium without affecting that of smooth muscle cells. IPC1 in non-diabetic hearts only, and IPC3 in all hearts, prevented the reduction in the vasodilation to 5-HT, suggesting that IPC can protect endothelial function in coronary arteries against the deleterious effect of ischaemia-reperfusion. A similar protective effect of IPC on endothelium-dependent acetylcholine-induced relaxation was observed in epicardial coronary arteries of normal dogs [16], and in left coronary arteries in vitro isolated from non-diabetic rats [17]. In contrast, in an anaesthetized open-chest canine model, IPC could not prevent the reduction in both endothelium-dependent and independent dilator responses observed after one hour coronary occlusion and 4 hours of reperfusion [36]. However, it is currently unclear whether this discrepancy is species-related or due to a different severity of the ischaemic insult.

4.2. Effect of preconditioning on myocardial recovery

In our study, 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. The results of the present study as well as others [6,7] showed that IPC enhances functional recovery in isolated non-diabetic rat

hearts. However, some reported no protective effect of IPC against myocardial dysfunction in isolated rat hearts [37]. Interestingly, recent studies have shown that aging hearts [38,39] as well as hearts from hypercholesterolaemic [40] and diabetic animals [22,23] do not benefit from IPC. In the present study, however, an improved post-ischaemic ventricular recovery with IPC was observed in the diabetic rat model.

4.3. Protective effect of exogenous adenosine

Adenosine has often been reported to be the endogenous mediator of the protection afforded by IPC [19,20]. We have recently reported that the adenosine-receptor antagonist, 8-phenyltheophylline, prevents the protective effect of IPC on the endothelial function in the isolated rat heart [18]. Therefore, we tested whether a reduced sensitivity to the cardioprotective effect of adenosine in diabetic hearts could explain the more extensive IPC required in these hearts. Two pre-treatment regimens with exogenous adenosine were compared: adenosine perfusion started either 15 min before ischaemia, being temporally equivalent to IPC1 with 5 min ischaemia and 10 min reperfusion, or 30 min before ischaemia, corresponding to IPC3 with 3 cycles of 5 min ischaemia and 5 min reperfusion. In accordance, a longer pre-treatment with adenosine was required in diabetic hearts (60 vs. 45 min) in order to prevent the ischaemia-induced reduction in the vasodilation to 5-HT. This difference is probably underestimated since we have recently observed that, in non-diabetic hearts, a 5 min treatment with 3 μM adenosine performed 10 min before ischaemia is sufficient to preserve the vasodilation to 5-HT ($-28 \pm 1\%$ vs. $-25 \pm 4\%$ for ischaemic and sham hearts, respectively, $n = 4$ per group). Thus, these data suggest that exogenous adenosine can mimic the protective effect of IPC on the endothelial function. In addition, the longer exposure to adenosine needed in diabetic hearts in order to observe a protective effect may explain the more extensive IPC required.

In contrast to the endothelial function, both the 45 min and 60 min adenosine pre-treatments were effective in improving the post-ischaemic recovery of dP/dt_{\max} and dP/dt_{\min} in diabetic hearts. However, adenosine could not significantly improve functional recovery in non-diabetic hearts. This is probably due to the fact that non-diabetic hearts recovered completely within the 20 min reperfusion, whereas functional recovery of diabetic hearts was blunted compared with control hearts. Therefore, the beneficial effect of adenosine will be more important in hearts with depressed ventricular function.

The mechanisms by which adenosine can exert a cardioprotective effect are numerous. Adenosine, released from ischaemic tissues and acting on A_1 receptors, can activate K_{ATP} channels via a G protein [41] and produce effects similar to those described for K_{ATP} channels. In the myocardium, activation of K_{ATP} channels possibly inhibits

ischaemic depolarisation, which could reduce Ca^{2+} entry via voltage-gated channels, resulting in a reduction in intracellular Ca^{2+} levels and decreased myocardial contractility [42]. Furthermore, recent studies have shown the presence of K_{ATP} channels in mitochondrial membranes. The role of these channels remains unknown, but it may maintain membrane polarity or even control mitochondrial Ca^{2+} concentration [42]. This control of intracellular Ca^{2+} concentration via K_{ATP} channel activation can prevent mitochondrial Ca^{2+} overload, a key player in myocardial ischaemic damage. Some evidences indicate that activation of K_{ATP} channels during ischaemia can also preserve the myocardial energy status [43,44]. Activation of PKC seems to be another major event in the cardioprotective effect of IPC in the rat heart [45,46]. Partial inhibition of PKC can provide additional protection, whereas complete inhibition blocks the protective effect of IPC [47]. Interestingly, adenosine and PKC can act in synergism to activate K_{ATP} channels in rabbit ventricular myocytes [48]. Liu et al. [20] have reported that adenosine acting through A_3 receptors can be a mediator of the ischaemic preconditioning. The physiological role of A_3 receptors is still poorly characterised, but they have recently been implicated as activators of mast cells [49]. According to this hypothesis mast cells would release mediators (histamine, leukotrienes, free radicals, thromboxanes, cytokines) during the preconditioning period (transient ischaemia) or during exogenous adenosine perfusion, producing little or no damage to myocytes, as these are washed away too rapidly. During the subsequent prolonged ischaemic insult, depleted mast cells could no longer release these deleterious mediators, resulting in a reduced myocardial or vascular damage [50]. It remains to be established whether these mechanisms can also explain the protective effect of IPC or exogenous adenosine perfusion on endothelial and myocardial functions.

There is a strong controversy whether the diabetic heart is more [28] or less [29] sensitive to ischaemic injury. In the present study, no marker of ischaemic injury such as CK release was measured. Therefore, the severity of the ischaemic injury in diabetic and normal hearts cannot be compared. However, post-ischaemic ventricular recovery was blunted in diabetic hearts. On the other hand, diabetic hearts needed more IPC periods and longer adenosine perfusion, compared with non-diabetic hearts, to achieve the same level of protection to the endothelial function. Although the exact mechanism of this difference remains unknown, several hypotheses can be suggested. First, the signalling pathways activated by endogenous or exogenous adenosine may be affected in diabetic coronary vessels. Since PKC and K_{ATP} channels are involved in the cardioprotective effect of IPC and adenosine, it is possible that an alteration of these mechanisms could explain the reduced sensitivity to the cardioprotective effect of IPC and adenosine observed in diabetic hearts. On the one hand, an attenuated PKC is unlikely, since many studies have re-

ported an increased PKC activity along with higher diacylglycerol levels in vascular tissues of diabetic animals [51,52]. Furthermore, dose-response curves to the PKC activator, phorbol 12-myristate 13-acetate, performed in diabetic hearts were found to be comparable with that of age-matched controls (Bouchard and Lamontagne, unpublished observation). On the other hand, reduced vascular responses to K_{ATP} channel activators in diabetes mellitus have been reported [53,54]. We have also observed a reduced coronary dilation with lemakalim in diabetic hearts (Bouchard and Lamontagne, unpublished observation). However, we cannot rule out that IPC in diabetic hearts involves activation of other pathways than those described for non-diabetic hearts.

These findings may have clinical implications. For example, during angioplasty or transplantation procedures, diabetic patients may need more ischaemic periods or a longer adenosine perfusion in order to achieve the same level of cardioprotection. However, it is certainly premature to extrapolate these findings to human beings and further experiments will be necessary.

In conclusion, these data suggest that ischaemic preconditioning or exogenous adenosine perfusion can afford protection to myocardial and endothelial function against subsequent ischaemic injury in diabetic hearts. However, to achieve the same degree of endothelial protection, preconditioning must be more extensive and adenosine perfusion period be longer in diabetic hearts, compared with non-diabetic hearts.

Acknowledgements

This project was supported by a grant from the Medical Research Council of Canada (MT-12260). JFB held a studentship from Association diabète Québec and from Merck-Frosst Canada.

References

- [1] Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986;74:1124–1136.
- [2] Reimer KA, Vander Heide RS, Jennings RB. Ischemic preconditioning slows ischemic metabolism and limits myocardial infarct size. *Ann NY Acad Sci* 1994;723:99–115.
- [3] Parratt JR. Protection of the heart by ischaemic preconditioning: mechanisms and possibilities for pharmacological exploitation. *Trends Pharmacol Sci* 1994;15:19–25.
- [4] Shiki K, Hearse DJ. Preconditioning of ischemic myocardium: reperfusion-induced arrhythmias. *Am J Physiol* 1987;253:H1470–H1476.
- [5] Piacentini L, Wainwright CL, Parratt JR. The antiarrhythmic effect of ischaemic preconditioning in isolated rat heart involves a pertussis toxin sensitive mechanism. *Cardiovasc Res* 1993;27:674–680.
- [6] Hagar JM, Hale SL, Kloner RA. Effects of preconditioning ischemia on reperfusion arrhythmias after coronary artery occlusion and reperfusion in the rat. *Circ Res* 1991;68:61–68.

- [7] Lasley RD, Anderson GM, Mentzer RM Jr., Ischaemic preconditioning enhance postischaemic recovery of function in the rat heart. *Cardiovasc Res* 1993;27:565–570.
- [8] Cave AC, Hearse DJ. Ischaemic preconditioning and contractile function: studies with normothermic and hypothermic global ischaemia. *J Mol Cell Cardiol* 1992;24:1113–1123.
- [9] Li Y, Kloner RA. Cardioprotective effects of ischaemic preconditioning are not mediated by prostanoids. *Cardiovasc Res* 1992;26:226–231.
- [10] Yellon DM, Alkhulaifi AM, Browne EE, Pugsley WB. Ischaemic preconditioning limits infarct size in the rat heart. *Cardiovasc Res* 1992;26:983–987.
- [11] Cohen MV, Liu GS, Downey JM. Preconditioning causes improved wall motion as well as smaller infarcts after transient coronary occlusion in rabbits. *Circulation* 1991;84:341–349.
- [12] Schwarz ER, Mohri M, Sack S, Arras M. Duration of infarct size limiting effect of ischemic preconditioning in the pig. *Circulation* 1991;84(Suppl. II):II–432. Abstract.
- [13] Yellon DM, Alkhulaifi AM, Pugsley WB. Preconditioning the human myocardium. *Lancet* 1993;342:276–277.
- [14] Pearson PJ, Schaff HV, Vanhoutte PM. Acute impairment of endothelium-dependent relaxations to aggregating platelets following reperfusion injury in canine coronary arteries. *Circ Res* 1990;67:385–393.
- [15] Van Benthuyzen KM, McMurtry IF, Horwitz LD. Reperfusion after acute coronary occlusion in dogs impairs endothelium-dependent relaxation to acetylcholine and augments contractile reactivity in vitro. *J Clin Invest* 1987;79:265–274.
- [16] DeFily DV, Chilian WM. Preconditioning protects coronary arteriolar endothelium from ischemia-reperfusion injury. *Am J Physiol* 1993;265:H700–H706.
- [17] Richard V, Kaeffer N, Tron C, Thuillez C. Ischemic preconditioning protects against coronary endothelial dysfunction induced by ischemia and reperfusion. *Circulation* 1994;89:1254–1261.
- [18] Bouchard J-F, Lamontagne D. Mechanisms of protection afforded by preconditioning to endothelial function against ischemic injury. *Am J Physiol* 1996;271:H1801–H1806.
- [19] Miura T, Iimura O. Infarct size limitation by preconditioning: its phenomenological features and the key role of adenosine. *Cardiovasc Res* 1993;27:36–42.
- [20] Liu GS, Richards SC, Olsson RA, Mullane K, Walsh RS, Downey JM. Evidence that the adenosine A₃ receptor may mediate the protection afforded by preconditioning in the isolated rabbit heart. *Cardiovasc Res* 1994;28:1057–1061.
- [21] Liu Y, Thornton JD, Cohen MV, Downey JM, Shaffer JR. Streptozotocin-induced non-insulin-dependent diabetes protects the heart from infarction. *Circulation* 1993;88:1273–1278.
- [22] Tosaki A, Pali T, Droy-Lefaix MT. Effects of Ginkgo biloba extract and preconditioning on the diabetic rat myocardium. *Diabetologia* 1996;39:1255–1262.
- [23] Tosaki A, Engelman DT, Engelman RM, Das DK. The evolution of diabetic response to ischemia/reperfusion and preconditioning in isolated working rat hearts. *Cardiovasc Res* 1996;31:526–536.
- [24] Tesfamariam B. Free radicals in diabetic endothelial cell dysfunction. *Free Radic Biol Med* 1994;16:383–391.
- [25] Abiru T, Watanabe Y, Kamata K, Kasuya Y. Changes in endothelium-dependent relaxation and levels of cyclic nucleotides in the perfused mesenteric arterial bed from streptozotocin-induced diabetic rats. *Life Sci* 1993;53:PL7–PL12.
- [26] Rodrigues B, McNeill JH. The diabetic heart: metabolic causes for the development of a cardiomyopathy. *Cardiovasc Res* 1992;26:913–922.
- [27] Miller TB. Cardiac performance of isolated perfused hearts from alloxan diabetic rats. *Am J Physiol* 1979;236:808–812.
- [28] Paulson DJ. The diabetic heart is more sensitive to ischemic injury. *Cardiovasc Res* 1997;34:104–112.
- [29] Feuvray D, Lopaschuk GD. Controversies on the sensitivity of the to ischemic injury is decreased. *Cardiovasc Res* 1997;34:113–120.
- [30] Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PA. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol Rev* 1994;46:157–193.
- [31] Mankad PS, Chester AH, Yacoub MH. 5-Hydroxytryptamine mediates endothelium dependent coronary vasodilatation in the isolated rat heart by the release of nitric oxide. *Cardiovasc Res* 1991;25:244–248.
- [32] Tesfamariam B, Cohen RA. Free radicals mediate endothelial cell dysfunction caused by elevated glucose. *Am J Physiol* 1992;263:H321–H326.
- [33] Oyama Y, Kawasaki H, Hattori Y, Kanno M. Attenuation of endothelium-dependent relaxation in aorta from diabetic rats. *Eur J Pharmacol* 1986;131:75–78.
- [34] Head RJ, Longhurst PA, Panek RL, Stitzel RE. A contrasting effect of the diabetic state upon the contractile responses of aortic preparations from the rat and rabbit. *Br J Pharmacol* 1987;91:275–286.
- [35] Smits P, Kapma J-A, Jacobs M-C, Lutterman J. Endothelium-dependent vascular relaxation in patients with type I diabetes. *Diabetes* 1988;42:148–153.
- [36] Bauer B, Simkhovich BZ, Kloner RA, Przyklenk K. Does preconditioning protect the coronary vasculature from subsequent ischemia/reperfusion injury. *Circulation* 1993;88:659–672.
- [37] Yang BC, Nicolini FA, Nichols WW, Mehta JL. Failure of brief ischemic episodes to protect against myocardial dysfunction caused by ischemia and reperfusion in isolated rat hearts. *Am Heart J* 1994;128:1192–1200.
- [38] Abete P, Ferrara N, Cioppa A, Ferrara P, Bianco S, Calabrese C, Cacciatore F, Longobardi G, Rengo F. Preconditioning does not prevent postischemic dysfunction in aging heart. *J Am Coll Cardiol* 1996;27:1777–1786.
- [39] Tani M, Saganuma Y, Hasegawa H, Shinmura K, Hayashi Y, Guo XD, Nakamura Y. Changes in ischemic tolerance and effects of ischemic preconditioning in middle-aged rat hearts. *Circulation* 1997;95:2559–2566.
- [40] Szilvassy Z, Ferdinandy P, Szilvassy J, Nagy I, Karcso S, Lonovics J, Dux L, Koltai M. The loss of pacing-induced preconditioning in atherosclerotic rabbits: role of hypercholesterolaemia. *J Mol Cell Cardiol* 1995;27:2559–2569.
- [41] Kirsch GE, Codina J, Birnbaumer L, Brown AM. Coupling of ATP-sensitive K⁺ channels to A₁ receptors by G proteins in rat ventricular myocytes. *Am J Physiol* 1990;259:H820–H826.
- [42] Grover GJ. Protective effects of ATP sensitive potassium channel openers in models of myocardial ischemia. *Cardiovasc Res* 1994;28:778–782.
- [43] Grover GJ, Newburger J, Sleph PG, Dzwonczyk S, Taylor SC, Ahmed SZ, Atwal KS. Cardioprotective effects of the potassium channel opener cromakalim: stereoselectivity and effects on myocardial adenine nucleotides. *J Pharmacol Exp Ther* 1991;257:156–162.
- [44] Hosoda H, Sunamori M, Suzuki A. Effect of pinacidil on rat hearts undergoing hypothermic cardioplegia. *Ann Thorac Surg* 1994;58:1631–1636.
- [45] Speechlydick ME, Mocanu MM, Yellon DM. Protein kinase C: its role in ischemic preconditioning in the rat. *Circ Res* 1994;75:586–590.
- [46] Mitchell MB, Meng X, Ao L, Brown JM, Harken AH, Banerjee A. Preconditioning of isolated rat heart is mediated by protein kinase C. *Circ Res* 1995;76:73–81.
- [47] Tosaki A, Maulik N, Engelman DT, Engelman RM, Das DK. The role of protein kinase in C ischemic/reperfused preconditioned isolated rat hearts. *J Cardiovasc Pharmacol* 1996;28:723–731.
- [48] Liu YG, Gao WD, O'Rourke B, Marban E. Synergistic modulation of ATP-sensitive K⁺ currents by protein kinase C and adenosine: implications for ischemic preconditioning. *Circ Res* 1996;78:443–454.

- [49] Ramkumar V, Stiles GL, Beaven MA, Ali H. The A₃ adenosine receptor is the unique adenosine receptor which facilitates release of allergic mediators in mast cells. *J Biol Chem* 1993;268:16887–16890.
- [50] Linden J. Cloned adenosine A₃ receptors: pharmacological properties, species differences and receptor functions. *Trends Pharmacol Sci* 1994;15:298–306.
- [51] Inoguchi T, Xia P, Kunisaki M, Higashi S, Feener EP, King GL. Insulin's effect on protein kinase C and diacylglycerol induced by diabetes and glucose in vascular tissues. *Am J Physiol* 1994;267:E369–E379.
- [52] Kunisaki M, Bursell SE, Umeda F, Nawata H, King GL. Normalization of diacylglycerol-protein kinase C activation by vitamin E in aorta of diabetic rats and cultured rat smooth muscle cells exposed to elevated glucose levels. *Diabetes* 1994;43:1372–1377.
- [53] Kamata K, Miyata N, Kasuya Y. Functional changes in potassium channels in aortas from rats with streptozotocin-induced diabetes. *Eur J Pharmacol* 1989;166:319–323.
- [54] Cameron NE, Cotter MA. Impaired contraction and relaxation in aorta from streptozotocin-diabetic rats: Role of polyol pathway. *Diabetologia* 1992;35:1011–1019.