Protection afforded by preconditioning to the diabetic heart against ischaemic injury

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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 followed by 20 min of reperfusion. Results: In the diabetic group (without ischaemia or IPC), infusion of 10 μM serotonin (5-HT), an endothelium-dependent, and 3 μM sodium nitroprusside (SNP), an endothelium-independent vasodilator, in the coronary bed preconstricted with 0.1 μM 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. IPC3 increased the recovery of myocardial recovery in both diabetic and non-diabetic hearts. Adenosine 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.

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 vessels [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
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 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\(_2\)PO\(_4\) 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 \(\mu\)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 \(\mu\)M serotonin (5-HT), whereas coronary smooth muscle function was evaluated using 3 \(\mu\)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 \(\mu\)M adenosine or vehicle starting after either a 20 min or a 35 min stabilisation period, in order to expose the hearts to either...
The presence of an interaction between the different groups, one way analysis of variance was 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-11α,9α-epoxymethano-prostaglandin F₂α) 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 μM 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 μM 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 μM, 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 μM 5-HT in sham hearts from diabetic rats (−25.2 ± 4.8%) was comparable to that of age-matched control rats (−25.2 ± 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) |
|-----------------|-----------------|-----------------|
| n               | Before U-46619  | After U-46619   |
| **IPC groups**  |                 |                 |
| **Non-diabetics** |                 |                 |
| Sham            | 8               | 10.10 ± 0.44    |
| Ischaemia       | 8               | 11.53 ± 0.63    |
| IPC1            | 8               | 11.53 ± 0.63    |
| IPC3            | 6               | 9.50 ± 0.60     |
| **Diabetics**   |                 |                 |
| Sham            | 8               | 9.34 ± 0.50     |
| Ischaemia       | 8               | 10.03 ± 0.62    |
| IPC1            | 6               | 8.73 ± 0.56     |
| IPC3            | 7               | 7.29 ± 0.65     |
| **Adenosine groups** |             |                 |
| **Non-diabetics** |                 |                 |
| 45 min perfusion| 8               | 8.95 ± 0.63     |
| 60 min perfusion| 4               | 9.72 ± 1.43     |
| **Diabetics**   |                 |                 |
| 45 min perfusion| 7               | 8.63 ± 0.62     |
| 60 min perfusion| 7               | 7.56 ± 0.66     |

Coronary resistance was calculated as perfusion pressure (mmHg)/perfusion flow (ml min⁻¹).
Values are means ± SEM.
*p < 0.05 compared with the corresponding ‘before U-46619’ value.

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diabetes 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 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/dtmax values measured before the 30 min partial ischaemia were 2405 ± 161 and 2307 ± 117 mmHg s⁻¹, and the dP/dtmin values 1695 ± 94 and 1702 ± 101 mmHg s⁻¹, for diabetic hearts and age-matched controls, respectively. Partial ischaemia was accompanied by a severe reduction in dP/dtmax (Fig. 3) in both diabetic and non-diabetic hearts. IPC3 improved dP/dtmax (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/dtmax 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/dtmax (Fig. 3) in diabetic and control rats. In all hearts studied, IPC1 was insufficient to improve ischaemic and post-ischaemic recovery of dP/dtmax (Fig. 3). The effect of ischaemia and reperfusion, with or without preconditioning, on dP/dtmin in both diabetic and non-diabetic hearts was superimposed with that on dP/dtmax (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...
just before the 30 min ischaemic period (−30.5 ± 2.5% in non-diabetic hearts (n = 12) and −28.1 ± 3.2% in diabetic hearts, p < 0.05, n = 14). The perfusion rate in non-diabetic hearts was 6.2 ± 0.3 ml min⁻¹ g⁻¹ (mean heart weight of 2.31 ± 0.09 g). In diabetic hearts, the perfusion rate was 7.29 ± 0.39 ml min⁻¹ g⁻¹ (mean heart weight of 2.14 ± 0.09 g). Infusion of U-46619 (0.1 μM, 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 10 μM 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 μM 5-HT in diabetic hearts (Fig. 4). Vasodilation to 3 μM 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 ± 64 mmHg s⁻¹, and the dP/dt min values 1574 ± 83 and 1392 ± 60 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 max 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), preconstriction of coronary arteries with U-
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 $dP/dt_{\text{max}}$ and $dP/dt_{\text{min}}$ 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 $K_{\text{ATP}}$ channels via a G protein [41] and produce effects similar to those described for $K_{\text{ATP}}$ channels. In the myocardium, activation of $K_{\text{ATP}}$ 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\(_{\text{ATP}}\) 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\(_{\text{ATP}}\) channel activation can prevent mitochondrial Ca\(^{2+}\) overload, a key player in myocardial ischaemic damage. Some evidence indicates that activation of K\(_{\text{ATP}}\) 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 synergy to activate K\(_{\text{ATP}}\) 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 ischaemia 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 degree of endothelial protection, presumably 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


