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Susceptibility to Ischemia-Induced Arrhythmias and the Effect of Preconditioning in the Diabetic Rat Heart

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Summary

Diabetic heart is suggested to exhibit either increased or decreased resistance to ischemic injury. Ischemic preconditioning suppresses arrhythmias in the normal heart, whereas relatively little is known about its effects in the diseased myocardium. Our objective was to investigate whether development of diabetes mellitus modifies the susceptibility to ischemia-induced arrhythmias and affects preconditioning in the rat heart. Following 1 and 9 weeks of streptozotocin-induced (45 mg/kg, i.v.) diabetes, the hearts were Langendorff-perfused at constant pressure of 70 mm Hg and subjected to test ischemia induced by 30 min occlusion of the left anterior descending (LAD) coronary artery. Preconditioning consisted of one cycle of 5 min ischemia and 10 min reperfusion, prior to test ischemia. Susceptibility to ischemia-induced arrhythmias was lower in 1-week diabetics: only 42 % of diabetic hearts exhibited ventricular tachycardia (VT) and 16 % had short episodes of ventricular fibrillation (VF) as compared to VT 100 % and VF 70 % (including sustained VF 36 %) in the non-diabetics ($P<0.05$). Development of the disease was associated with an increased incidence of VT (VT 92 %, not significantly different from non-diabetics) and longer total duration of VT and VF at 9-weeks, as compared to 1-week diabetics. Preconditioning effectively suppressed arrhythmias in the normal hearts (VT 33 %, VF 0 %). However, it did not provide any additional antiarrhythmic protection in the acute diabetes. On the other hand, in the preconditioned 9-weeks diabetic hearts, the incidence of arrhythmias tended to decrease (VT 50 %, transient VF 10 %) and their severity was reduced. Diabetic rat hearts are thus less susceptible to ischemia-induced arrhythmias in the acute phase of the disease. Development of diabetes attenuates increased ischemic tolerance, however, diabetic hearts in the chronic phase can benefit more from ischemic preconditioning, due to its persisting influence.

Key words

Experimental diabetes • Myocardial ischemia • Preconditioning • Arrhythmias • Rat heart

Introduction

Diabetic patients are more prone to congestive heart failure and/or ischemic heart disease including myocardial infarction, and severe ventricular arrhythmias are frequently encountered. The above complications can develop even in the absence of coronary artery disease and are attributed to the development of diabetic

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cardiomyopathy that has been described in various clinical and experimental settings (Fein *et al.* 1980, Dhalla *et al.* 1985, Dubrey *et al.* 1994). Contractile dysfunction as well as rhythm disorders are caused by alterations in the cell membrane ion transport systems responsible for the maintenance of Na^+, K^+, and Ca^{2+} homeostasis (reviewed by Dhalla *et al.* 1998). Numerous animal studies revealed that diabetes causes myocardial remodeling and defects in the cell sarcolemmal and sarcoplasmic membranes resulting in abnormal function of ion transport systems and impaired Ca^{2+} handling (Ganguly *et al.* 1983, Makino *et al.* 1987, Khandoudi *et al.* 1990, Pierce *et al.* 1990, Lee *et al.* 1992). Increased intracellular calcium itself has been found to inhibit the function of the Na^+ pump (Breier *et al.* 1998) and thus to aggravate calcium overload of the cell, as well as to depress the transport and/or utilization of glucose (Sulová *et al.* 1998). From this point of view, it appears that diabetic hearts should be more vulnerable to ischemia/reperfusion. However, experimental data are controversial and suggest that the sensitivity of diabetic hearts to ischemia may be altered (Feuvray and Lopaschuk 1997). Thus, the susceptibility to arrhythmias in the diabetic hearts has been found to be enhanced (Hekimian *et al.* 1985), unchanged (Beatch and McNeill 1988) or reduced (Kusama *et al.* 1992).

Prolonged ischemia induces profound alterations in heart metabolism, function and ultrastructure. On the contrary, brief episodes of ischemia have been found to protect the hearts of different species (dogs, rabbits, rats, guinea pigs) against subsequent prolonged ischemia, an adaptive phenomenon termed as ischemic preconditioning (Murry *et al.* 1986). Protection can be manifested by a reduced size of infarction (Liu and Downey 1993), improved postischemic contractile recovery (Cave 1995), as well as by suppression of malignant ischemia-induced arrhythmias (Vegh *et al.* 1992). The studies of preconditioning are usually performed on normal healthy animals, whereas relatively little is known whether mechanisms of adaptation of the heart to ischemia are modified in the diseased myocardium. Some authors suggest that preconditioning is "a healthy heart phenomenon" (Tosaki *et al.* 1996), while others have also observed preconditioning-induced protection in diabetic hearts (Tatsumi *et al.* 1998).

The present study was designed to investigate whether response to ischemia and ischemic preconditioning are modified during the development of diabetes mellitus. We have chosen a rat model of streptozotocin-induced diabetes of different duration, and ischemia-induced malignant arrhythmias in the isolated heart as the main end-point of protection. Our results suggest that diabetic rats are less sensitive to ischemia-induced arrhythmias in the early period of the disease. Development of diabetic cardiomyopathy partially attenuates this protective effect. However, these hearts can benefit more from preconditioning than in the acute phase.

Methods

Animals

Male Wistar rats (250-300 g body weight), fed a standard diet and tap water *ad libutum*, were used. All studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No 85-23, revised 1996).

Diabetes was induced by a single i.v. injection of streptozotocin (45 mg/kg), whereas control animals received an equal amount of a vehicle (1 mM citrate buffer). Development of the disease was confirmed by enhanced blood glucose levels. Following one week of diabetes duration (acute phase) and nine weeks (chronic phase), the diabetic animals, as well as the controls were sacrificed and all experiments were performed on isolated perfused hearts.

Perfusion technique

Rats were anesthetized (sodium pentobarbitone, 60 mg/kg, i.p.) and given heparin (500 IU, i.p.). Hearts were rapidly excised, placed in ice-cold perfusion buffer, cannulated *via* the aorta and perfused in the Langendorff mode at a constant perfusion pressure of 70 mm Hg and at 37 °C. The perfusion solution was a modified Krebs-Henseleit buffer gassed with 95 % O_2 and 5 % CO_2 (pH 7.4) containing (in mM): NaCl 118.0, KCl 3.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, NaH_2PO_4 1.18, $CaCl_2$ 2.5, glucose 11.1. Reduced potassium and increased calcium concentrations were used to promote arrhythmogenesis during ischemia. The solution was filtered through a 5 μm porosity filter (Millipore) to remove contaminants.

An epicardial electrogram (EG) was registered by means of two stainless steel electrodes attached to the apex of the heart and the aortic cannula and continuously recorded (Mingograph ELEMA-Siemens, Solna, Sweden). Heart rate was calculated from the EG. Coronary flow was measured by a timed collection of

coronary effluent. Left ventricular pressure was measured by means of a latex water-filled balloon inserted into the left ventricle *via* the left atrium (adjusted to obtain end-diastolic pressure of 5-7 mm Hg) and connected to a pressure transducer (P23 Db Pressure Transducer, Gould Statham Instruments, USA). The left ventricular pressure (LVDP, systolic minus diastolic pressure), maximum rates of pressure development and fall (+dP/dt and –dP/dt) as the indexes of contraction and relaxation, as well as heart rate, coronary flow and pressure-rate product (PRP; LVDP x HR) were used to assess cardiac function.

Arrhythmias were analyzed in accordance with The Lambeth Conventions (Walker *et al.* 1988). In this study we focused on measuring the incidence of ventricular tachycardia (VT) and fibrillation (VF) as well as of their duration. VT was defined as a run of four or more consecutive ectopic beats. VF lasting more than 2 min was considered as sustained. The severity of arrhythmias was quantified by a scoring system, where hearts with premature ventricular beats only were given a score of 1, bigeminy/salvos a score of 2, VT a score of 3, transient VF a score of 4 and a score of 5 was given to the hearts with sustained VF. Each individual heart was assigned a number corresponding to the most severe type of arrhythmia observed and the scores were used for group analysis of the severity of arrhythmias.

Experimental protocols

After 30-min equilibration, all hearts were randomly assigned to the following protocols:

1. Test ischemia

After additional 15-min perfusion, both, diabetic and control hearts (n = 12-13 per group) were subjected to a test ischemic challenge. Regional ischemia was induced by a ligature placed around the left anterior descending (LAD) coronary artery close to its origin, both ends of which were threaded through a traction-type plastic occluder and clamped. Coronary artery occlusion lasted 30 min, and was followed by a release of clamping to permit reperfusion. The efficacy of occlusion and reperfusion was confirmed by a fall in coronary flow at the onset of ischemia of about 40 % and its recovery upon reperfusion. Further verification was performed by dye exclusion technique and measurement of an ischemic zone size (Ravingerová *et al.* 1995).

2. Ischemic preconditioning

After equilibration, diabetic and control hearts (n=12–13 per group) were subjected to one cycle of ischemic preconditioning consisting of 5 min ischemia and 10 min reperfusion, prior to test ischemia as described previously (Ravingerová *et al.* 1997, Okruhlicová *et al.* 2000).

Statistics

Data were expressed as means ± S.E.M. One-way analysis of variance (ANOVA) was first applied to test for any significant differences in normally distributed variables among the groups. If the differences were established, individual groups were compared using the unpaired Student's *t*-test. Non-Gaussian distributed variables (incidences of VT and VF) were compared using Fisher's exact test. Differences were considered significant when $P<0.05$.

Results

Development of diabetes

After one and nine weeks of diabetes duration, blood glucose levels significantly increased to 17.4±0.7 and 23.8±0.9 mM, as compared to 5.74±0.1 and 5.43±0.26 mM in age-matched controls ($P<0.05$), respectively. In the diabetic rats, body weight loss was already observed after one week of the disease and even more markedly after nine weeks (Table 1). Heart weight

Table 1. Body and heart weight of rats after 1 and 9 weeks of streptozotocin-induced diabetes mellitus.

Parameters	1 week		9 weeks	
	Control	Diabetic	Control	Diabetic
BW (g)	329±6	286±14*	362±10	259±22*
HW (mg)	800±50	875±30	920±10	838±16*
HW/BW (mg/g)	2.43±0.1	3.06±0.3*	2.54±0.1	3.2±0.2*

*Data are means ± S.E.M., n = 24-26 in each group. BW – body weight, HW – heart weight, HW/BW – relative heart weight. Significant differences: *P<0.05 (diabetic animals vs. corresponding age-matched non-diabetic controls).*

was moderately decreased only in the chronic phase. However, relative heart weight (heart weight/body weight ratio) was increased at 1 and 9 weeks of diabetes as compared to the respective age-matched controls.

Table 2. Pre-ischemic parameters of cardiac function after 1 and 9 weeks of streptozotocin-induced diabetes mellitus in isolated rat hearts.

Parameters	1 week		9 weeks	
	Control	Diabetic	Control	Diabetic
HR	300±11	266±9*	320±8	203±8*#
CF	13.6±1.5	11.9±0.8	12.2±0.6	8.6±0.4*#
LVDP	94±4	75±6.3*	90±2	79±3.9*
+dP/dt	3320±180	2407±280*	2881±187	1918±130*#
–dP/dt	1959±160	1577±150*	1826±50	1291±77*#
PRP	27570±1300	20534±2070*	25800±1250	15720±1600*#

*Data are means ± S.E.M., n=24-26 in each group. HR – heart rate (beats/min), CF – coronary flow (ml/min), LVDP – left ventricular developed pressure (mm Hg), +dP/dt, –dP/dt – maximum rates of pressure development and fall, respectively (mm Hg/s), PRP – pressure-rate product (mm Hg x beats/min). Significant differences: *P<0.05 (diabetic hearts vs. corresponding age-matched non-diabetic controls), #P<0.05 (chronic diabetic vs. acute diabetic hearts).*

Cardiac function in the diabetic hearts

Evaluation of cardiac function in the Langendorff preparation before the onset of ischemia revealed a gradual and significant reduction of heart rate and coronary flow in the course of the disease (Table 2).

Preischemic values of LVDP were similar in both diabetic groups and moderately decreased, as compared to the controls. The rates of contraction and relaxation (+dP/dt and –dP/dt), as well as PRP were consistently lower in both diabetic groups, especially in the chronic one, indicating impaired myocardial performance due to a gradual development of heart failure.

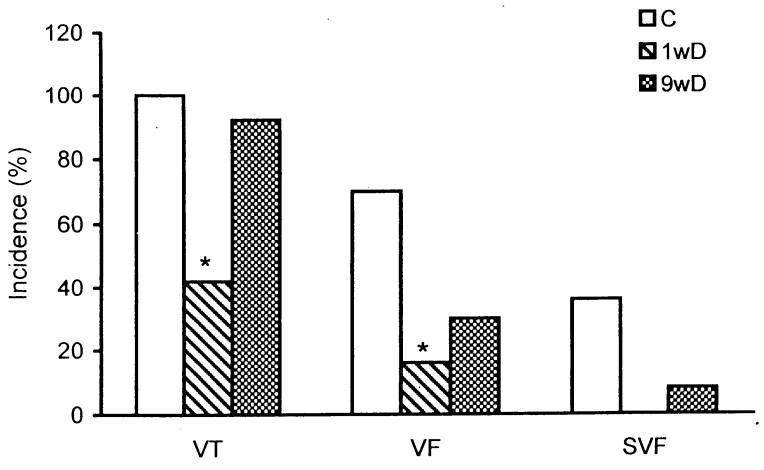

Fig. 1. *Effect of acute and chronic streptozotocin-induced diabetes on susceptibility to ischemic arrhythmias in isolated rat hearts. In the control group, the data were pooled from the non-diabetics age-matched with 1-week and 9-weeks diabetics. C – non-diabetic control hearts (open columns, n=24), 1wD – acute diabetic hearts (hatched columns, n=12), 9wD – chronic diabetic hearts (cross-hatched columns, n=13). VT – ventricular tachycardia, VF – ventricular fibrillation, SVF – sustained ventricular fibrillation (lasting >2 min). Significant differences: * P<0.05 (diabetic hearts vs. non-diabetic controls).*

Susceptibility to ischemia-induced arrhythmias

In this model, LAD coronary artery occlusion produced an ischemic zone (area at risk) amounting to approximately 43 % of total ventricular mass (similar in all groups of non-diabetic and diabetic hearts), and severe ventricular arrhythmias peaked between 10 and 20 min of ischemia. In the non-diabetic controls, VT was observed in all the hearts (Fig. 1), and 70 % of the hearts exhibited VF (total incidence including transient VF, as well as sustained VF that occurred in 36 % of the hearts). In the hearts of 1-week diabetic rats, the incidence of VT was lower (42 % *vs.* 100 % in the controls) and only transient VF occurred in 16 % of the hearts, whereas sustained VF was completely abolished ($P<0.05$). In the chronic phase of the disease, alterations in cardiac function were accompanied by an exacerbation of arrhythmias: the incidence of VT increased and appeared to be similar to that in the non-diabetic controls. The total incidence of VF (non-sustained and sustained) was also moderately increased to 30 % including sustained VF in 8 % of the hearts. Thus the differences between the diabetic and non-diabetic control hearts observed at one week disappeared at nine weeks (Fig. 1).

Not only the incidence, but also the duration of arrhythmias was affected by diabetes. The total duration of both VT and VF was significantly shorter in acute diabetic hearts than in the corresponding controls (Table 3). At nine weeks, the total duration of VT and VF increased in comparison to that in the 1-week diabetic hearts, however, it was still shorter than in the age-matched controls.

Table 3. Effect of acute and chronic diabetes on the duration of ventricular arrhythmias in the preconditioned (PC) and non-preconditioned (non-PC) isolated rat hearts.

Groups	n	Duration of VT and VF (s)	
		Non-PC	PC
Controls	47	657 ± 25	17.5 ± 11$^{\#}$
1-week diabetics	25	11.4 ± 5.5*	133 ± 53*$^{\#}$
9-weeks diabetics	23	46.5 ± 12.5*†	8.3 ± 4$^{\#\dagger}$

*Data are means ± S.E.M. In the control group, the data were pooled from the non-diabetic hearts age-matched with 1-week and 9-weeks diabetic hearts. VT – ventricular tachycardia, VF – ventricular fibrillation. Significant differences: *P<0.05 (diabetic animals vs. corresponding non-diabetic controls), $^{\#}$P<0.05 (preconditioned vs. non-preconditioned hearts), † P<0.05 (chronic diabetic vs. acute diabetic hearts).*

Severity of arrhythmias in the 1-week and 9-weeks diabetic hearts, evaluated by means of arrhythmia score, corresponded to the above findings (Fig. 2, open bars). Severity of arrhythmias was significantly lower in the 1-week diabetic group than in the controls (2.25±0.35 vs. 4.1±0.3, $P<0.05$) reflecting an enhanced resistance of the hearts to ischemia in the acute phase of the disease (Fig. 2, middle). Development of diabetes was associated with an increase in severity (Fig. 2, right), although it was still lower than in the non-diabetic controls (3.25±0.2 vs. 4.1±0.3, $P<0.05$).

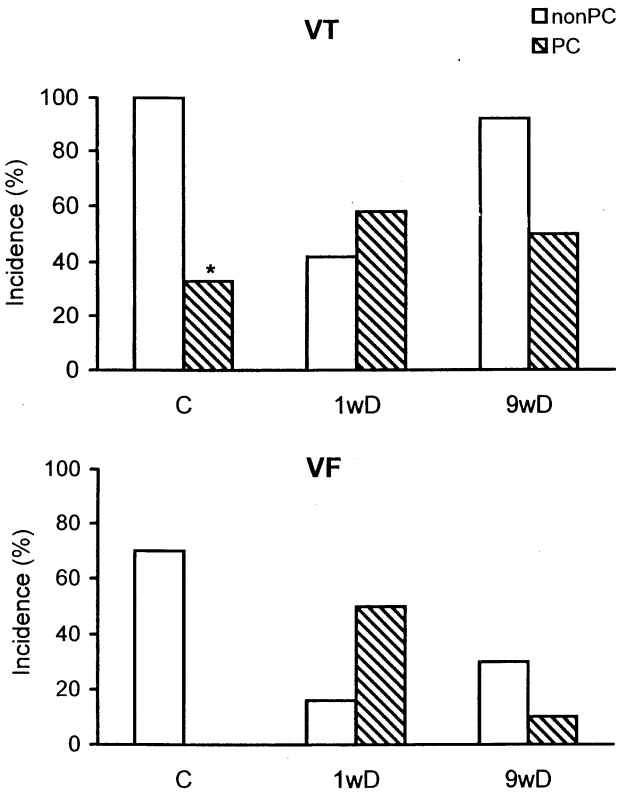

Fig. 2. *Effect of diabetes and preconditioning (PC) on the severity of arrhythmias evaluated by arrhythmia score in isolated rat hearts. C – controls, 1wD – acute diabetes, 9wD – chronic diabetes. Data are means of 10-24 experiments in each group ± S.E.M. Open columns - non-preconditioned hearts, hatched columns – preconditioned hearts. Significant differences: * P<0.05 (preconditioned vs. non-preconditioned hearts), $^{\#}$ P<0.05 (diabetic hearts vs. non-diabetic controls).*

Effect of ischemic preconditioning on susceptibility to arrhythmias

In the non-diabetic hearts, ischemic preconditioning markedly suppressed the incidence and severity of arrhythmias (Fig. 3). The incidence of VT was

decreased to 33 % and VF was totally abolished (P<0.05). In the acute diabetic hearts, this intervention failed to induce any additional antiarrhythmic protection, and there was a tendency to enhance arrhythmias. The occurrence of VT (Fig. 3, top) did not differ significantly from that in the non-preconditioned diabetic hearts (58 % vs. 42 %, NS). Total incidence of VF (Fig. 3, bottom) was 50 % (due to sustained VF that occurred in 25 % of the hearts; not shown). This resulted in a prolonged total duration of arrhythmias as compared to the non-preconditioned hearts (Table 3).

In the 9-week diabetic hearts, preconditioning was more effective than in 1-week diabetic hearts. Incidence of VT was reduced to 50 % (Fig. 3, top). In contrast to the ineffective suppression of VF in the acute diabetic hearts, only a short episode of transient VF occurred in one of ten hearts (Fig. 3, bottom). The total duration of arrhythmias in these hearts was also affected by preconditioning and was significantly shorter than in the non-preconditioned hearts (Table 3).

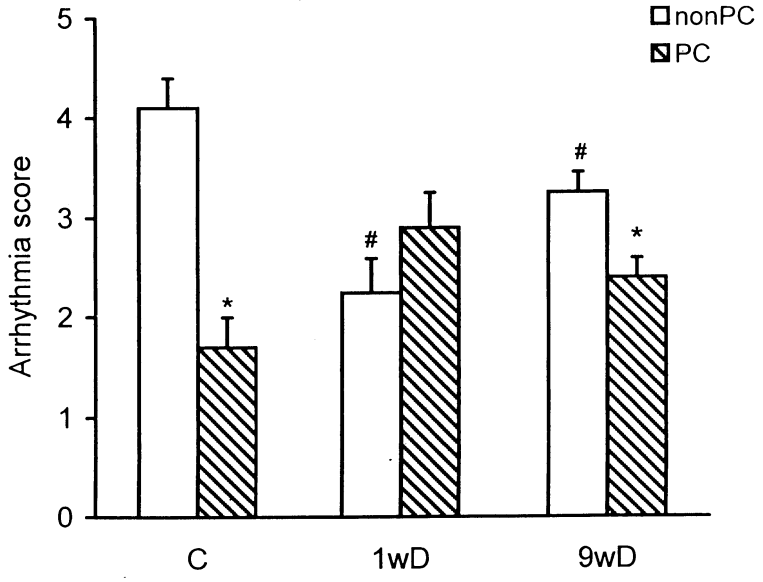

Fig. 3. *Effect of preconditioning (PC) on the susceptibility to ischemic arrhythmias in isolated rat hearts with acute and chronic diabetes mellitus. C – controls, 1wD – acute diabetes, 9wD – chronic diabetes. Abbreviations as in Figure 1. Data are from 10-24 experiments in each group. Open columns – non-preconditioned hearts, hatched columns – preconditioned hearts. Significant differences: * P<0.05 (preconditioned vs. non-preconditioned hearts).*

Figure 2 (hatched bars) demonstrates the effect of preconditioning on the severity of arrhythmias. Preconditioning reduced the severity of arrhythmias in the controls (from the score 4.1±0.3 to 1.7±0.3, P<0.05). At one week of diabetes, the severity of arrhythmias in the preconditioned diabetic hearts did not differ from that in the non-preconditioned ones (2.9±0.5 vs. 2.3±0.4, NS). On the other hand, at 9 weeks of diabetes, the hearts benefited more from preconditioning and severity of arrhythmias was reduced (from the score 3.3±0.2 to 2.4±0.3, P<0.05).

Discussion

Although a large body of evidence has accumulated in clinical studies of diabetic cardiomyopathy suggesting an increased risk of myocardial infarction and a higher rate of mortality, experimental results have reported either increased or decreased susceptibility to ischemic injury under different experimental conditions. Furthermore, it is not quite clear whether protective mechanisms are operating in the pathologically altered myocardium. The results of present study demonstrate that diabetic hearts in the early phase of the disease are more resistant to ischemia-induced severe ventricular arrhythmias than the non-diabetic controls. One week of diabetes (with significantly elevated blood glucose levels) already caused myocardial dysfunction manifested by a significant reduction of heart rate and cardiac performance (Table 2). Moderately decreased LVDP and lower heart rate can account for reduced rates of contraction and relaxation in the diabetic hearts (+/– dP/dt). However, in these hearts, the incidence and total duration of VT and VF was lower, and sustained VF was totally abolished during 30 min regional ischemia, the intervention which induces a high incidence of ventricular arrhythmias in the normal heart (Fig. 1). One of the major determinants of arrhythmogenesis is the size of the ischemic area (Curtis 1998). However, we can disregard this factor since there were no differences in the size of the ischemic area (area at risk) between the groups. Tosaki *et al.* (1996) demonstrated a lower

incidence of reperfusion-induced arrhythmias in the rat heart in the early phase of diabetes associated with lower intracellular accumulation of Ca^{2+} that has been proposed to play a major role in arrhythmogenesis (Levy 1989). The resistance to arrhythmias caused by high calcium in the diabetic rat heart can be explained by the alterations in the properties of L-type Ca^{2+} channels (Lee *et al.* 1992) and reduced Ca^{2+} influx into the cells.

Electrophysiological mechanisms underlying antiarrhythmic protection might involve alterations in the outward potassium currents in the diabetic myocardium, reduced to a different extent in the epi- and endocardial layers, the latter being suggested to attenuate dispersion of refractoriness as a substrate for re-entry arrhythmias (Shimoni *et al.* 1995). Moreover, ATP-sensitive potassium channels (K_{ATP}) in the diabetic cardiomyocytes have been found to be much more sensitive and open at higher levels of ATP than in non-diabetic controls (Smith and Wahler 1996). Activation of K_{ATP} channels is considered as one of the mechanisms protecting against arrhythmias related to triggered activity due to enhanced Ca^{2+} influx (Spinelli *et al.* 1991). Recently, it has been demonstrated that chronic myocardial hypoxia renders the rat heart more resistant to ischemia-induced arrhythmias (Asemu *et al.* 1997) and K_{ATP} channels localized in mitochondria appear to be involved in this protection (Asemu *et al.* 1999). Permanent tissue hypoxia is a common feature of the diabetic myocardium (for review see Dhalla *et al.* 1998). It is conceivable that similar mechanisms of intrinsic cardioprotection might be activated in the diabetic heart as well. Thus, the early period of diabetes seems to be associated with adaptation to the disease when endogenous cardioprotective mechanisms are successfully counteracting metabolic disorders leading to functional deterioration and dysrhythmias.

Development of the disease leads to a gradual attenuation of antiarrhythmic protection due to persisting metabolic alterations. Massive intracellular accumulation of toxic intermediates of fatty acid metabolism (e.g. acylcarnitine and long chain acyl-CoA) within sarcolemma has been suggested to alter biophysical properties of cell membranes and cause electrophysiological derangements (such as cell-to-cell uncoupling) as well as deterioration of membrane-bound ion transporting enzymes (McHowat *et al.* 1993). Inhibition of Na^+/K^+- and Ca^{2+}-ATPases in the cardiac sarcolemma of the diabetic hearts (Makino *et al.* 1987) might enhance the susceptibility to ischemia- and reperfusion-induced arrhythmias (Curtis and Hearse 1989). Nevertheless, although the incidence of VT and VF in this study increased in the chronic phase (Fig. 1), their duration and severity still remained lower than in the controls indicating partial preservation of protective mechanisms (Table 3, Fig. 2). Some authors found a suppression of the initial protection with the duration of the disease associated with an enhanced calcium accumulation in the myocardium (Tosaki *et al.* 1996), while others have stressed the increased resistance to ischemic injury induced by coronary artery occlusion even after longer duration of the disease (Hadour *et al.* 1998). These conflicting data may partially be explained by species differences, severity of different protocols (utilizing regional, global, low-flow ischemia or hypoxia) and the choice of the endpoints studied (reviewed by Paulson 1997). It is suggested that processes related to the alterations in glucose metabolism and intracellular pH regulation might be responsible for the reduced sensitivity of the diabetic hearts to severe zero-flow ischemia (Feuvray and Lopaschuk 1997). A decreased accumulation of glycolytic products on the one hand and a reduced activity of Na^+/H^+ exchanger on the other hand (Khandoudi *et al.* 1990, Pierce *et al.* 1990), may account for a lower Na^+ gain during ischemia and reduced Ca^{2+} entry *via* Na^+/Ca^{2+} exchange upon reperfusion. This is consistent with our previous observation of an increased resistance to calcium overload in the diabetic rat heart (Ravingerová *et al.* 1996).

In the present study, ischemic preconditioning by one cycle of ischemia/reperfusion effectively suppressed ischemia-induced arrhythmias in the normal hearts, while it did not confer any additional protection in the acute phase of diabetes. It seems that enhanced resistance to ischemia in the early period of diabetes might "mask" the effect of classical ischemic preconditioning. Both conditions may share some common mechanisms, such as activation and translocation of protein kinase C (Mitchell *et al.* 1995), as well as opening of K_{ATP} channels (Tan *et al.* 1993), the mechanisms of cardioprotection that are activated in the diabetic myocardium even in the early phase (Malhotra *et al.* 1997, Smith and Wahler 1996). It is also possible that diabetes interferes with the mechanisms of preconditioning and affects the threshold for antiarrhythmic protection.

In the chronic phase of the disease, the heart benefited more from preconditioning since arrhythmias were reduced in a way comparable with the preconditioned non-diabetic controls (Figs 2 and 3, Table 3), indicating that the potential for ischemic preconditioning was still preserved. A cardioprotective

effect of preconditioning has also been demonstrated in open-chest rats with coronary artery occlusion even after much longer duration of diabetes (Liu *et al.* 1993). This differs from the study by Tosaki *et al.* (1996) who demonstrated a lack of preconditioning protection in the chronic phase of diabetes in the isolated rat heart subjected to global ischemia and reperfusion. However, the latter can be attributed to the differences between the mechanisms of ischemia- and reperfusion-induced events (Curtis and Hearse 1989, Hearse 1996) and to different models utilized in our and their studies (Langendorff-perfused hearts and regional ischemia vs. working hearts and global ischemia).

In chronic diabetes, glucose transport and utilization are severely impaired (Rodrigues *et al.* 1995). On the other hand, inhibition of glucose uptake can mimic ischemic preconditioning and salvage normal myocardium (Goto *et al.* 1995). It is also known that brief ischemic episodes deplete glycogen stores and thus reduce the rate of production of ATP and of acid glycolytic metabolites (Murry *et al.* 1986), whereas glycogen recovery is associated with a loss of protection (Wolfe *et al.* 1993). Recently, it has been demonstrated that in the diabetic hearts, despite a higher glycogen content, preconditioning induces a more effective depletion of myocardial glycogen stores and a lower lactate production during ischemia contributing thus to a greater myocardial protection than in the normal hearts (Tatsumi *et al.* 1998). So far, it is difficult to differentiate whether it is a real effect of ischemic preconditioning itself, or of still persisting diabetes-induced intrinsic cardioprotection, or a combination of both, that may account for the protective effect.

In conclusion, the results of our study show that the early period of diabetes is associated with an increased resistance of the rat heart against ischemia-induced arrhythmias. This protective effect is partially attenuated in the chronic phase of the disease associated with deterioration of heart function. Preconditioning does not confer any additional protection in the early phase, whereas the hearts can benefit more in the chronic phase of diabetes, due to a persisting potential for preconditioning. The exact mechanisms of this effect, however, require further investigation.

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