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Hypercholesterolemia abrogates an increased resistance of diabetic rat hearts to ischemia-reperfusion injury

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Abstract

Both, diabetes mellitus (DM) and hypercholesterolemia (HCH) are known as risk factors of ischemic heart disease, however, the effects of experimental DM, as well as of HCH alone, on ischemia/reperfusion-induced myocardial injury are not unequivocal. We have previously demonstrated an enhanced resistance to ischemia-induced arrhythmias in rat hearts in the acute phase of DM. Our objectives were thus to extend our knowledge on how DM in combination with HCH, a model that is relevant to diabetic patients with altered lipid metabolism, may affect the size of myocardial infarction and susceptibility to arrhythmias. A combination of streptozotocin (STZ; 80 mg/kg, i.p.) and the fat–cholesterol diet (1% cholesterol, 1% coconut oil; FCHD) was used as a double-disease model mimicking DM and HCH simultaneosly occurring in humans. Following 5 days after STZ injection and FCHD leading to increased blood glucose and cholesterol levels, anesthetized open-chest diabetic, diabetic–hypercholesterolemic (DM–HCH) and age-matched control rats were subjected to 6-min ischemia (occlusion of LAD coronary artery) followed by 10 reperfusion to test susceptibility to ventricular arrhythmias in the *in vivo* experiments and to 30-min ischemia and subsequent 2-h reperfusion for the evaluation of the infarct size (IS) in the Langendorff-perfused hearts. The incidence of the most life-threatening ventricular arrhythmia, ventricular fibrillation, was significantly increased in the DM–HCH rats as compared with non-diabetic control animals (100% vs. 50%; $p < 0.05$). Likewise, arrhythmia severity score (AS) was significantly higher in the DM–HCH rats than in the controls (4.9 ± 0.2 vs. 3.5 ± 0.5; $p < 0.05$), but was not increased in the diabetic animals (AS 3.7 ± 0.9; $p > 0.05$ vs. controls). Diabetic hearts exhibited a reduced IS ($15.1 \pm 3.0\%$ of the area at risk vs. $37.6 \pm 2.8\%$ in the control hearts; $p < 0.05$), however, a combination of DM and HCH increased the size of myocardial infarction to that observed in the controls. In conclusion, HCH abrogates enhanced resistance to ischemia-reperfusion injury in the diabetic rat heart.

Key words: arrhythmias, experimental diabetes, hypercholesterolemia, infarction, myocardial ischemia-reperfusion injury

Introduction

Although epidemiological and clinical data have clearly demonstrated that diabetic patients are more prone to ischemic heart disease [1], experimental studies have revealed controversies in the sensitivity of the diabetic heart to ischemia/reperfusion injury. The unchanged [2–4], increased [5, 6] and even decreased [4, 7, 8] susceptibility

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to myocardial ischemia/reperfusion injury (MIRI) have been reported in different animal models. Our previous studies have also shown that experimental diabetes does not result in an increased vulnerability to MIRI [9–11]. Several mechanisms have been proposed to explain a lower sensitivity to ischemia/reperfusion in the diabetic heart. The alterations in the intracellular pH, a decreased clearence of protons via Na/H exchanger, a decreased rate of glycolysis in the diabetic myocardium may represent the main possible mechanisms of this attenuated response to ischemia/reperfusion injury [12–14]. Further explanations, such as severity of ischemic protocol (global zero-flow versus low-flow ischemia), the presence of the metabolic substrate in the perfusion medium and the differences related to the experimental models utilized in the studies (open-chest animals or isolated hearts) can be also taken into consideration. The severity and type of the induced diabetes, as well as the differences in the animal species can also modify myocardial sensitivity to ischemia/reperfusion injury [6, 12]. Thus, dogs with diabetes are supposed to be more sensitive to ischemia and develop larger infarcts than normal dogs [15], whereas in the diabetic rabbits, as well as in the diabetic rats, infarct size (IS) tends to be smaller than in the non-diabetic animals [7, 16].

Hypercholesterolemia (HCH), as well as DM, is associated with an increased risk of coronary artery disease and progression of myocardial infarction [17]. Different from clinical trials, the results of many experimental studies have not shown unequivocally an increased vulnerability of the hearts of hypercholesterolemic animals to ischemic/reperfusion injury. Acutely hypercholesterolemic animals have exhibited an enhanced susceptibility to injury, whereas animals exposed to HCH for longer periods of time have shown a higher resistance to MIRI [13, 18, 19]. Besides the duration of the disease, differences between animal species may account for different myocardial response to injury [13]. Thus, low-density lipoprotein receptor-deficient hypercholesterolemic mice fed a normal diet are supposed to be less vulnerable to MIRI [20].

Our previous studies, both *in vitro* and *in vivo*, have been performed to test myocardial ischemic tolerance in the acute and chronic phase of streptozotocin-induced diabetes mellitus (DM) [9–11]. However, little is known whether and how other simultaneously occurring pathological condition affects a susceptibility to MIRI in the diabetic hearts. Therefore, the present study was designed to extend our knowledge on the effect of the combined injury on the outcome of MIRI in two different experimental settings. Diabetic–hypercholesterolemic (DM–HCH) model was chosen to explore a response of the hearts to MIRI because of its relevance to a clinical situation when diabetic patients suffer from both, altered lipid metabolism and simultaneously occurring ischemic heart disease. Susceptibility to life-threatening ventricular arrhythmias and the size of myocardial infarction served as the end-points of injury in both *in vivo* and *in vitro* experiments. Our results suggest that DM–HCH rat hearts in the acute phase of the combined disease exhibit a higher sensitivity to MIRI-induced injury.

Materials and methods

Animals

Male Wistar rats (250–300 g body weight), fed a standard diet and tap water ad libitum, were used for all studies. The experimental protocols 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) and with the approval by the local Ethics Committee.

Induction of simultaneously occurring diabetes mellitus and hypercholesterolemia

A modified double-disease model of the DM–HCH rats developed by Jiao *et al*. [21] and Kusunoki *et al*. [22] was used in this study. DM was induced by a single intraperitoneal injection of streptozotocin (STZ, 80 mg/kg) diluted in buffer solution (0.05 mol/l citrate buffer, 0.1 mol/l NaCl; pH 4.5). HCH was induced by an application of the fat–cholesterol diet (FCHD; 1% cholesterol, 1% coconut oil). Rats were fasted overnight before application of streptozotocin. Immediately after the application of STZ rats were allowed to drink 5% glucose solution for the first 24 h and afterwards they were fed the FCHD (20 g/day). Development of DM and HCH was confirmed by enhanced blood glucose and total cholesterol levels, as well as by the total cholesterol contents in liver (see Results).

Following 5 days after the induction of the mentioned pathological states, diabetic and DM–HCH rats, as well as the age-matched control ones were randomly assigned to the following protocols.

Surgical procedure of ischemia-reperfusion injury in the open-chest model

Animals were anesthetized with pentobarbital (45 mg/kg, i.p.). Acute coronary occlusion and reperfusion were performed according to Lepran and Szekeres [23]. After tracheal cannulation and thoracotomy, the ligature was placed loosely around the left coronary artery. The heart was set back into thoracic cavity and the rats started to be artificially ventilated with room air by a pump (CIIC, USA) using a stroke volume of 1.2 ml/100 g and a rate of 50–60 breaths/min. After 10 min stabilization period, left coronary artery was occluded for 6 min followed by 10 min

reperfusion. The heart action was continuously recorded by electrocardiograph (Seiva, Czech Republic) during all phases of the experiment.

Surgical procedure of ischemia-reperfusion injury in the in vitro model

Perfusion technique

Rats were anesthetized (pentobarbital 60 mg/kg, i.p.) and heparinized (500 IU, i.p.). Hearts were rapidly excised, cannulated via the aorta and placed into the Langendorff setup (ADInstruments, Germany) for perfusion at a constant perfusion pressure of 70 mm Hg and at 37 °C. Perfusion solution was a modified Krebs-Henseleit buffer gassed with 95% O_2 and 5% CO_2 (pH 7.4) containing (in mmol/l): NaCl 118.0; KCl 3.2; $MgSO_4$ 1.2; $NaHCO_3$ 25.0; KH_2PO_4 1.18; $CaCl_2$ 2.5; glucose 7.0. 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. Left ventricular pressure was measured by means of a non-elastic water-filled balloon inserted into the left ventricle via the left atrium (adjusted to obtain end-diastolic pressure of 5–7 mm Hg and deflated at the onset of ischemia) and connected to a pressure transducer (MLP844 Physiological Pressure Transducer, ADInstruments). Left ventricular developed pressure (LVDP, systolic minus diastolic pressure), maximal rates of pressure development and fall (+dP/dtmax and −dP/dtmax) as the indexes of contraction and relaxation were monitored during stabilization pre-ischemia period, while heart rate (HR) (derived from EG) and coronary flow (CF) were continuously recorded until the end of reperfusion. Heart function was analyzed using PowerLab/8SP Chart 5 software (ADInstruments). Similar technique as in the *in vivo* study was utilized to induce regional ischemia [9]. Efficacy of LAD coronary artery occlusion was verified by an immediate fall in CF. After the end of ischemia, the snare was released to permit reperfusion of the ischemic tissue that was indicated by an increase in CF and appearance of reperfusion arrhythmias. Ischemia was maintained for 30 min followed by reperfusion lasting for 2 h.

Quantification of arrhythmias

In the *in vivo* experiments, we focused on the incidence of potentially lethal ventricular arrhythmias, such as ventricular tachycardia (VT) and fibrillation (VF), that were analyzed according to the guidelines for the study of ischemia and reperfusion arrhythmias known as The Lambeth Conventions [24]. VT was defined as a run of four or more consecutive ectopic beats. In addition, severity of arrhythmias was quantified by a scoring system. Each individual heart was evaluated by means of a 6-point arrhythmia score and an assigned number corresponded to the most severe type of arrhythmia observed in that heart. Scores were used for the group analysis of the severity of arrhythmias [25].

Infarct size determination

At the end of reperfusion, the hearts were arrested in diastole with 0.25 mg verapamil (Isoptin, Knoll). The area at risk (AR) and the IS were determined as described previously [10] by double staining with 5% potassium permanganate and with 1% 2,3,5-triphenyltetrazolium chloride (Sigma, USA) dissolved in 0.1 M phosphate buffer (pH 7.4). The hearts were cut perpendicularly to the long axis of the ventricle into the 1 mm thick slices and stored overnight in 10% neutral formaldehyde solution. The IS, size of the AR and size of the left ventricle (LV) were determined by a computerized planimetric method. The IS was normalized to the size of AR (IS/AR) and the size of AR was normalized to the LV size (AR/LV).

Measurement of biochemical parameters

In parallel subsets of experiments, the samples of blood for estimation of glucose (GLU) and total cholesterol (TCHOL) were collected from the abdominal aorta of the animals from all groups. Plasma was prepared by centrifugation at 5000 rpm for 15 min. GLU and TCHOL were measured enzymatically using a commercial assay kit (Spinreact, USA) and bioanalyzer ELISA 200 (USA).

Liver samples were homogenized and lipids were extracted in the mixture containing ethanol and ether using the method of Brown *et al*. [26] as modified by Bligh and Dyer [27]. TCHOL in the extracts was measured enzymatically using a commercial assay kit (Spinreact, USA) and spectrophotometer Varian DMS 300 (USA).

Statistical evaluation

The biochemical parameters, arrhythmia score, IS data and values of parameters of myocardial function were expressed as means ± SEM for the number (n) of animals in the group. One-way ANOVA and subsequent Student-Newman-Keuls test were used for comparison of differences in parametric variables among the groups. The incidence of arrhythmias was expressed as percentage and compared by using the 2 × 2 chi-square test. Differences between the groups were considered significant when $p < 0.05$ and noted in figures and tables.

Results

Development of diabetes and hypercholesterolemia

Development of DM and HCH was confirmed by significantly increased plasma glucose and cholesterol levels, as well as total cholesterol content in liver (Table 1).

Table 1. Plasma glucose (GLU), cholesterol (TCHOL) and liver total cholesterol (TCHOL) levels in the control (C), diabetic (DM) and diabetic–hypercholesterolemic (DM–HCH) rats

Group	Plasma		Liver
	GLU mmol/l	TCHOL mmol/l	TCHOL mg/g
C (n = 10)	5.5 ± 0.7	1.5 ± 0.1	2.6 ± 0.2
DM (n = 7)	17.2 ± 0.7 **	1.8 ± 0.3	2.8 ± 0.9
DM–HCH (n = 10)	18.0 ± 2.4 ††	2.9 ± 0.5 † /	7.4 ± 1.0 ††† ///

The data are expressed as means ± SEM for 7–10 measurements.
**$p < 0.01$ diabetic animals versus controls.
$^{\dagger}p < 0.05$; $^{\dagger\dagger}p < 0.01$ and $^{\dagger\dagger\dagger}p < 0.001$ diabetic–hypercholesterolemic animals versus controls.
$^{/}p < 0.05$; $^{///}p < 0.001$ diabetic–hypercholesterolemic animals versus diabetics.

After 5 days, plasma glucose levels in the diabetic group were significantly increased to 17.2 ± 0.7 as compared with 5.5 ± 0.7 in the non-diabetic controls animals ($p < 0.01$), whereas cholesterol levels amounted to 1.8 ± 0.3 mmol/l in the diabetic animals and to 1.5 ± 0.1 mmol/l in the age-matched controls. On the other hand, simultaneous DM and FCHD resulted in significantly increased plasma levels of cholesterol as compared with both, the control and the diabetic group (2.9 ± 0.5 mmol/l; $p < 0.05$ vs. controls and diabetics). Likewise, significantly increased total cholesterol content in liver was observed in the DM–HCH rats in comparison with the non-diabetic controls and the diabetic animals (7.4 ± 1.0 mmol/l vs. 2.6 ± 0.2 mmol/l and 2.8 ± 0.9 mmol/l; respectively; $p < 0.001$).

Characteristics of isolated hearts

The values of HR, LVDP, $+(dP/dt)_{max}$, $-(dP/dt)_{max}$ and CF in the control non-diabetic, diabetic and DM–HCH groups are summarized in Table 2. In the hearts of the diabetic animals, the HR was significantly decreased as compared with the non-diabetic controls (239 ± 11 vs. 260 ± 5.5; $p < 0.05$). Reduced HR was also observed in the DM–HCH group (Table 2). There were no significant differences in the values of other parameters between the groups at baseline before the induction of ischemia. At the end of 2-h reperfusion, HR tended to decrease similarly in all groups, and CF ranged between 75% and 83% of baseline values.

Susceptibility to ventricular arrhythmias in the open-chest rats

The incidence of potentially lethal arrhythmias in the control, diabetic and DM–HCH rats is shown in Fig. 1. The

Table 2. Preischemic values of parameters of myocardial function in Langendorff-perfused rat hearts after 5 days of streptozocin and fat–cholesterol-diet-induced double disease

Parameters	Control (n = 15)	Diabetic (n = 8)	Diabetic–hypercholesterolemic (n = 8)
HR (beats/min)	260 ± 5.5	239 ± 11*	234 ± 18†
CF (ml/min)	11.9 ± 1.1	10.3 ± 0.8	12.7 ± 1.3
LVDP (mm Hg)	75.4 ± 7.5	75.6 ± 3.9	81.5 ± 8.3
$+(dP/dt)_{max}$ (mm Hg/s)	2226 ± 236	2149 ± 159	2161 ± 276
$-(dP/dt)_{max}$ (mm Hg/s)	1398 ± 141	1405 ± 51	1514 ± 195

Values are means ± SEM. Data from the two corresponding control groups were pooled.
HR – heart rate, CF – coronary flow, LVDP – left ventricular developed pressure, +(dP/dt) max and −(dP/dt) max – maximum rates of pressure development and fall, respectively.
*$p < 0.05$ diabetic animals versus controls.
$^{\dagger}p < 0.05$ diabetic–hypercholesterolemic animals versus controls.

results demonstrate that the incidence of these ventricular tachyarrhythmias was greater in the DM–HCH rats than in the diabetics alone and control animals. VT occurred in 90% of DM–HCH rats as compared with 86% and 80% of diabetic and age-matched control rats, respectively. All rats subjected to simultaneously occurring diabetes and HCH for 5 days exhibited episodes of VF, whereas the total incidence of VF (both, transient and sustained VF) was significantly lower in the control animals (VF 50%; $p < 0.05$ vs. DM–

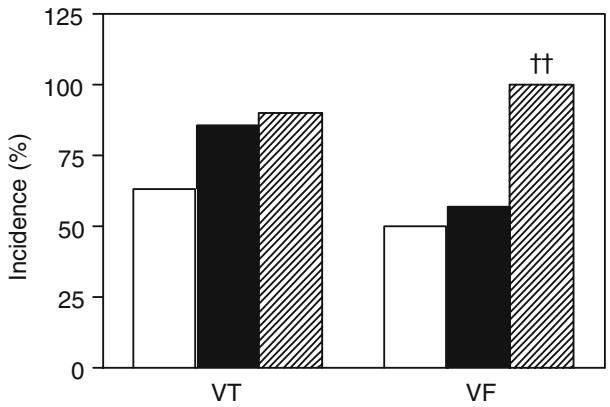

Fig. 1. Effect of simultaneously occurring acute diabetes mellitus and hypercholesterolemia on the incidence of ventricular arrhythmias in the open-chest rats. Ischemia was induced by 6 min occlusion of LAD coronary artery and followed by 10 min reperfusion. Empty bars – non-diabetic control hearts; hatched bars – diabetic hearts, filled bars – diabetic–hypercholesterolemic hearts, VT – ventricular tachycardia, VF – ventricular fibrillation. Values are % of incidence (n = 7–10 per group). Data from the two corresponding control groups were pooled. $^{\dagger\dagger}p < 0.01$ diabetic–hypercholesterolemic animals versus controls.

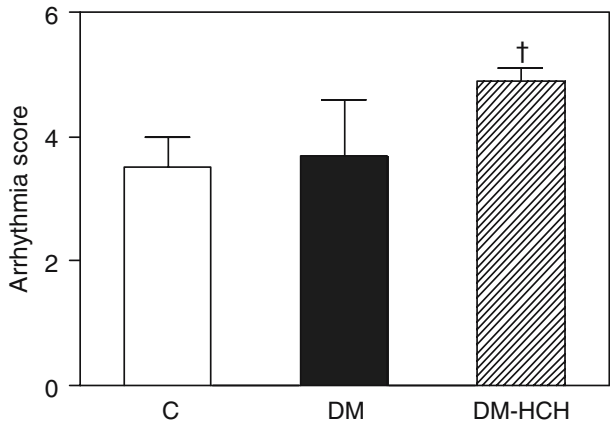

Fig. 2. Effect of simultaneously occurring acute diabetes mellitus and hypercholesterolemia on arrhythmia severity score in the open-chest rats. C – non-diabetic control rats; DM – diabetic rats, DM–HCH – diabetic–hypercholesterolemic rats. Values are means ± S.E.M (7–10 experiments per group). Data from the two corresponding control groups were pooled. $^{\dagger}p < 0.05$; diabetic-hypercholesterolemic animals versus controls.

HCH rats). In the diabetic group, VF occurred in 57% of animals that did not differ from its incidence in the controls and tended to be lower than in the DM–HCH group. Severity of arrhythmias (arrhythmia score) in the group of DM–HCH animals was significantly increased as compared with the control ones (AS 4.9 ± 0.2 vs. 3.5 ± 0.5; $p < 0.05$), while in the diabetic group it did not differ from the controls (AS 3.7 ± 0.9; Fig. 2).

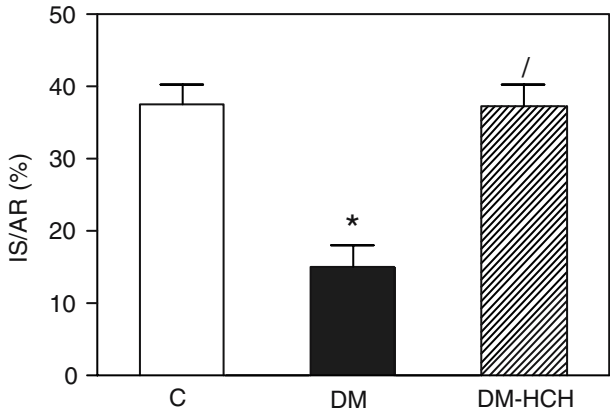

Fig. 3. Effect of simultaneously occurring acute diabetes mellitus and hypercholesterolemia on the size of myocardial infarction. Ischemia was induced by 30 min occlusion of LAD coronary artery and followed by 2 h reperfusion. Infarct size (IS) was normalized to the size of area at risk (AR). C – non-diabetic control hearts; DM – diabetic hearts, DM–HCH – diabetic–hypercholesterolemic hearts. Values are means ± S.E.M. (6–8 experiments per group). Data from the two corresponding control groups were pooled. $^{*}p < 0.05$ diabetic animals versus controls; $^{/}p < 0.05$; diabetic–hypercholesterolemic animals versus diabetics.

Table 3. Effect of STZ-induced acute diabetes and simultaneously occurring diabetes and HCH on the size of myocardial infarction

Group	AR/LV(%)	IS/LV(%)
Control (n = 15)	49.9 ± 1.9	18.6 ± 1.3
Diabetic (n = 8)	47.0 ± 2.4	6.4 ± 1.2**
Diabetic–hypercholesterolemic (n = 8)	45.9 ± 1.2	28.2 ± 9.6//

The infarct size (IS) and the size of area at risk (AR) were normalized to the size of left ventricle (LV).
Values are means ± S.E.M. Data from the two corresponding control groups were pooled.
$^{**}p < 0.01$ diabetic animals versus controls.
$^{//}p < 0.01$ diabetic–hypercholesterolemic animals versus diabetics.

Infarct size

The IS normalized to the size of the AR was significantly lower in the diabetic hearts than in the control ones (IS/AR 15.1 ± 3.0% vs. 37.3 ± 3.1%; $p < 0.05$; Fig. 3). In the hearts of rats with both pathological states, DM and HCH, IS/AR was increased to the level similar to that in the controls (IS/AR 37.6 ± 2.8%). When the size of infarction was normalized to the size of the left ventricle (IS/LV), the deleterious effect of HCH on the diabetes-induced reduction in the size of infarction was also evident (IS/LV were 18.6 ± 1.3%, 6.4 ± 1.2% and 28.2 ± 9.6% in the control, diabetic and DM–HCH groups, respectively; Table 3). The size of AR normalized to the size of the left ventricle (AR/LV) did not differ between the groups (49.9 ± 1.9% in the control, 47.0 ± 2.4% in the diabetic and 45.9 ± 1.2% in the DM–HCH group, respectively; Table 3).

Ventricular arrhythmias in the isolated hearts

Ventricular tachycardia occurred in 73% of the control hearts and its incidence was reduced to 25% in the diabetic hearts ($p < 0.05$ vs. controls). In the DM–HCH group, the incidence of VT was increased as compared with the diabetic group and reached the level of controls (Fig. 4).

Discussion

Myocardial ischemic/reperfusion injury has been described in numerous clinical settings. The mechanisms of injury that develop during ischemia and subsequent reperfusion have been extensively studied and are relatively well defined in healthy and/or mono-diseased myocardium, e.g., during diabetes, HCH, hypertension and heart failure [13]. A substantial deal of studies has been performed to investigate a severity of ischemic injury in various diabetic alone and hypercholesterolemic alone animal models. However, to the

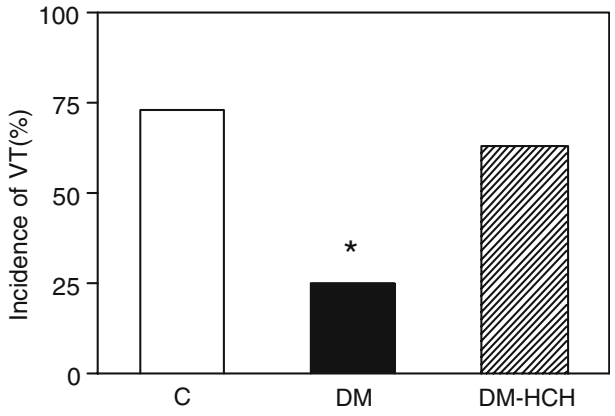

Fig. 4. Effect of simultaneously occurring acute diabetes mellitus and hypercholesterolemia on the incidence of ventricular tachycardia (VT) in the isolated hearts. Abbreviations as in Fig. 2. Values are % of incidence (n = 6–8 per group). Data from the two corresponding control groups were pooled. *$p < 0.05$ diabetic animals versus controls.

best of our knowledge, none of the studies has been focused on the effect of the combination of the above-mentioned risk factors, which often occur simultaneously in humans, on the outcome of MIRI.

The results of studies dealing with MIRI in both insulin-dependent and non-dependent diabetic rats are controversial. The phase of the disease is one of many factors, which determine the outcome of MIRI [4]. In the previous study [10], we have observed that open-chest rats in the acute phase of streptozotocin-induced DM exhibit lower sensitivity to ischemia/reperfusion-induced myocardial infarction. Likewise, the results of our study using isolated rat hearts indicated that acute diabetics are also more resistant to ischemia-induced ventricular arrhyhmias [11]. Tosaki *et al*. [4] have also demonstrated a reduction in the incidence of reperfusion-induced arrhythmias, as well as improved functional recovery upon reperfusion in the rat heart in the early phase of diabetes. In the present study in the Langendorff model, we have confirmed our previous results obtained in the model *in vivo* that the diabetic heart has a smaller size of infarction. Although the experimental protocol in the isolated hearts was not designed to evaluate arrhythmogenesis, we observed a significantly lower incidence of VT in the diabetic hearts in this model. In contrast, the experiments in the open-chest model were aimed to study arrhythmias. In accordance, the protocol of MIRI in this *in vivo* model resulted, in comparison with the *in vitro* study, in much higher incidence of lethal arrhythmias including VF that were not exacerbated in the diabetic animals. This is in concert with the results of our previous study [10] demonstrating that in the open-chest rat model of MIRI, susceptibility to ventricular tachyarrhythmias is not modified in the acute phase of diabetes. Taken together, our results show that the capability to tolerate ischemia-induced necrotic changes and susceptibility to malignant arrhythmias is not affected in the STZ diabetic rats. These findings indicate that early period of the disease is associated with activation of adaptive mechanisms, which successfully counteract metabolic disorders leading to irreversible cell damage and arrhythmias.

Enhanced resistance to ischemic injury observed in the STZ-induced diabetic heart can be considered as an alternative form of intrinsic cardioprotection analogous to that induced by short-term adaptive phenomenon of ischemic preconditioning in the normal heart, in which numerous metabolic stimuli, in particular those related to oxidative stress and increased intracellular calcium signaling can trigger protection against ischemia/reperfusion [28].

Activation and translocation of protein kinase C (PKC) appears to be a key player in various cardioprotective phenomena including classical ischemic preconditioning [29], delayed protection induced by pretreatment with catecholamines [30] and long-term cardioprotection conferred by adaptation to chronic hypoxia [31]. Oxidative stress and myocardial hypoxia is a common feature of a number of chronic processes including diabetic cardiomyopathy, and PKC activation is also known to occur in the diabetic myocardium even in the early phase of the disease [32]. Moreover, its translocation has been shown to mediate cardioprotection in the STZ-induced diabetic rat heart, whereas PKC inhibition abolished an increased resistance to MIRI in the diabetic hearts [33, 34]. Another mechanism of increased resistance to injury induced by hyperglycemia has been suggested by Schaffer *et al*. [35], who demonstrated that glucose treatment attenuated hypoxia-induced calcium overload of the cells by upregulation of cardioprotective antiapoptotic factor Bcl-2 and enhancing the inactivation of proapoptotic Bad factor.

To investigate an influence of acute chemically-induced HCH on the progression and outcome of MIRI in rats is complicated for the reason of their relative resistance to cholesterol feeding. Thus, rabbits and mice have been most often used in such experiments. The animals with HCH in acute phase of the disease are supposed to be more sensitive to MIRI. It has been observed that IS of rabbit hearts after short-term (3–4 days) cholesterol diet was increased approximately by 100%. [19, 36]. Furthermore, IS in wild-type mice with short-time (2 weeks) HCH does not change, although it markedly enhances in LDL receptor-deficient mice [20]. However, it seems that prolonged exposure of hearts to high circulating cholesterol levels may probably lead to induction of endogenous cardioprotective mechanisms. HCH developing during 12 weeks resulted in significant decrease in IS in both wild-type and LDL r−/− mice [20]. Improvement of cardiac contractile recovery have been also shown in rabbits fed 2% cholesterol diet for

5–16 weeks compared with rabbits fed the same diet for 2–3 weeks [37]. On the other hand, HCH in these animals may induce a significant increase of life-threatening ventricular arrhythmias [38]. These opposite findings may be related to a reduction of myocardial gluthathion levels after 2 weeks of persisting HCH and its elevation after 12 weeks [20]. Taken together, short-term hyperlipidemia indepedently from the development of coronary atherosclerosis, deteriorates the outcome of myocardial ischemia-reperfusion injury.

On the basis of the above-mentioned findings it is conceivable to propose that the acute diabetic rats exhibit decreased susceptibility to MIRI, while the animals with the acute phase of HCH are more sensitive to this injury. However, a response to MIRI in hearts of animals with both present pathological conditions is relatively unknown. On the other hand, it is well documented that an enhanced resistance to MIRI in the normal preconditioned myocardium may be abolished by pathological conditions, such as HCH [39]. Thus, 16 weeks high cholesterol diet in rabbits blunted the IS limiting effect of ischemic preconditioning [40].

Therefore, the present study was designed to investigate how acute DM and HCH together may affect susceptibility of rat hearts to MIRI in the *in vivo* and *in vitro* settings. The study using the open-chest animals demonstrated that 5 days of simultaneously occurring DM and HCH increased myocardial sensitivity to life-threatening ventricular arrhythmias. Indeed, the severity of injury in the open-chest DM–HCH rats assessed by arrhythmia score was higher. Although the *in vitro* experiments focused on the IS determination, maintenance of both pathological conditions for 5 days did not aggravate irreversible necrotic processes, HCH completely reversed IS-limiting effect observed in the hearts from the diabetic rats. It seems that the differences in the pathogenesis of arrhythmias and myocardial infarction, as well as between the experimental models may account for the above-mentioned discrepancy. Thus, further studies are required to explore the specific mechanisms of this phenomenon.

These are the novel and original findings since any similar study related to MIRI has not yet been performed in animals with acute chemically-induced diabetes and HCH. The only available data are those by Hoshida *et al*. [41] who studied the outcome of MIRI in genetic non-insulin-dependent Zucker diabetic fatty (ZDF) rats fed a cholesterol diet. Despite some differences in the experimental protocol (duration of ischemia and reperfusion, types of DM and diet, duration of HCH), these DM–HCH rats also developed an increased sensitivity to MIRI, which was associated with enhanced P-selectin expression, increased neutrophil accumulation in ischemic tissue and impaired endothelium-dependent relaxation.

Conclusion

This study demonstrated that DM–HCH rats exhibit an enhanced sensitivity to arrhythmias induced by ischemia-reperfusion injury and the extent of necrotic changes in their myocardium is higher than in the hearts of rats with diabetes alone. It appears thus that HCH attenuates cardioprotective effects of acute experimental diabetes and increases vulnerability of the heart to MIRI. On the other hand, while early period of simultaneously occuring diabetes and HCH is associated with an increased susceptibility to severe ventricular arrhythmias, the response of DM–HCH myocardium to irreversible cell damage is similar to that in the healthy heart. The above discrepancy might be related to the factors, such as the differences in the pathogenesis of myocardial infarction and arrhythmias, as well as in the design of experimental protocols. Additional studies are required to elucidate the mechanisms of a differential response of the double-diseased DM–HCH animals to MIRI.

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