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Original articles

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FLUIDISING EFFECT OF RESORCYLIDENE AMINOGUANIDINE ON SARCOLEMMAL MEMBRANES IN STREPTOZOTOCIN-DIABETIC RATS: BLUNTED ADAPTATION OF DIABETIC MYOCARDIUM TO Ca^{2+} OVERLOAD

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The "remodelling" of cardiac sarcolemma in diabetes is believed to underlie the reduced sensitivity of diabetic hearts due to their overload with extracellular calcium. Along with a non-enzymatic glycosylation and the free radical-derived glycoxidation of sarcolemmal proteins there is ongoing reduction in cardiomyocyte membrane fluidity, the modulator of cardiac sarcolemmal functioning. Aminoguanidine derivatives, that inhibit glycation and glycoxidation, might suppress myocardium "remodelling" occurring in diabetic heart. To verify this hypothesis, we studied physical parameters of cardiac sarcolemma from the streptozotocin-induced diabetic rats (45 $mg.kg^{-1}$ i.m.) treated with resorcylidene aminoguanidine (RAG, 4 or 8 $mg.kg^{-1}$ i.m.). The treatment with RAG not only completely abolished protein glycation and a generation of free oxygen species ($p<0.001$) in treated diabetic animals, but also considerably attenuated the decrease in sarcolemmal membrane fluidity ($p<0.001$). In diabetic animals the "normalization" of the sarcolemmal membrane fluidity was accompanied by the vastly increased susceptibility of diabetic hearts to be overload with external calcium. We concluded that the decreased fluidity of the sarcolemmal membrane, apparently linked to the excessive glycation of sarcolemmal membrane proteins, might be intimately connected with the adaptation mechanism(s) that are likely to develop in diabetic heart to protect it against the overload with external calcium.

Key words: *heart sarcolemma, glycation, membrane fluidity, DPH fluorescence anisotropy, resorcylidene aminoguanidine*

INTRODUCTION

The substantial bulk of evidence, which has been accumulated for years on the impact of chronic hyperglycaemia encountered in diabetes mellitus* (DM) on long-term diabetic complications, points to the crucial role of protein non-enzymatic glycosylation (glycation) and a persisting oxidative stress (1). The enhanced protein glycation and formation of early glycation products in diabetes is intimately associated with the process of "glycoxidation" and generation of reactive carbonyl intermediates involved in the formation of both protein crosslinks and advanced glycation end-products (2). As the latter accumulate, they participate in structural and functional alterations of diverse intracellular structures (3-5), including cellular membranes of diabetic myocardium (6). A variety of the diabetes-driven dysfunctions of cardiomyocytes, which may involve altered function of membrane receptors and transport systems, abnormal cell homeostasis with impaired signal transduction and Ca^{2+}-handling, and/or decreased cardiomyocyte Ca^{2+}-sensitivity (increased Ca^{2+}-resistance) (6-9), converge to what is termed the "remodelling" of diabetic heart.

The observations that some glycoxidation-related events might relate to adaptation mechanisms of diabetic heart to maintain its functioning even under unfavourable metabolic conditions (7), have prompted the search and interest in the use of chemical modulators with anti-glycation and/or antioxidation activities, which might be useful in "normalising" the distorted function of diabetic myocardium. Accordingly, the studies pointing to the successful use of aminoguanidine derivatives – the agents with recognised anti-glycation and antioxidation properties – to attenuate the impact of diabetic hyperglycaemia on the impaired function of diabetic heart appeared to be the convincing arguments in favour of the indisputable role of DM-induced glycoxidation in the "remodelling" of cardiac sarcolemma (SL) (6, 10). Two selected compounds of this class, resorcylidene aminoguanidine and pyridoxal aminoguanidine, have been shown to be effective not only as the anti-glycation agents, but also to show antioxidant activity. Whereas the former was apparently achieved by the selective blocking of the reactive carbonyls on early glycation products, the antioxidant activity might be deduced from their chemical structure (11-13). Moreover, both these aminoguanidine derivatives have the ability to fluidise cellular membrane lipid bilayer, and the fluidising properties seem to be independent of their anti-glycation and antioxidation effects (14, 15).

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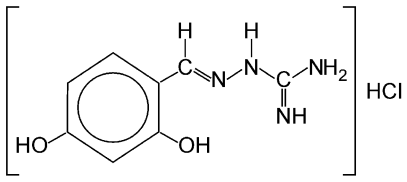
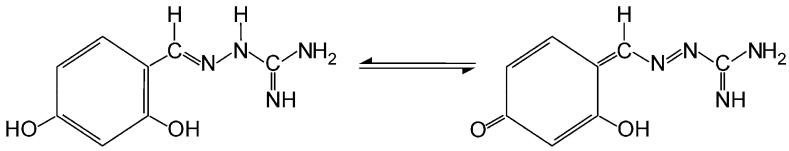
* Abbreviations: AGEs – advanced glycation end products; C – control; D – diabetic; DM – diabetes mellitus; DPH -1,6-diphenyl-1,3,5-hexatriene, fluorescent probe; RAG – (b-resorcylidene aminoguanidine; SL – heart sarcolemma(l).



β-Resorcylidene aminoguanidine • HCl (RAG)
molecular formula: $C_8H_{10}N_4O_2$ • HCl
molecular weight: 230.65

formation of RAG quinoid structure



In our previous study, we have shown that the RAG-mediated reduction in non-enzymatic glycosylation may be associated with lower calcium tolerance of diabetic hearts (6). The role of altered dynamic properties of cardiac sarcolemmal membranes in the functioning of myocardium in diabetes remains elusive. However, the pieces of evidence accumulated hitherto may point to the adaptation of cardiac SL to diabetes (8, 9). We raised the hypothesis that the reduction in protein glycation and glycoxidation and the modulation of cardiac sarcolemmal membrane fluidity by RAG may restore normal function of diabetic myocardium and have an impact on functional "remodelling" of cardiac sarcolemma in experimental diabetes. In this study, we discuss the diabetes-induced changes in SL membrane fluidity in the context of their propensity to improve the survival of a diabetic heart. We report that the decreased fluidity of the sarcolemmal membrane may relate to some adaptation mechanisms in "remodelled" diabetic heart, which might be protective against the overload with external calcium.

MATERIALS AND METHODS

Chemicals

All chemicals were purchased from Lachema (Brno, Czech Republic) unless otherwise stated. Tris [2-amino-2-(hydroxymethyl)-1,3-propanediol] and streptozotocin were from Sigma Chemicals Co. (USA) and Interdep™ (insulin) was from Slovakofarma (Bratislava, Slovakia). 1,6-diphenyl-1,3,5-hexatriene (DPH) was purchased from Serva GmbH (Germany).

Synthesis of resorcylidene aminoguanidine

The chemical synthesis of β-resorcylidene aminoguanidine.HCl (RAG, mol. wt. 230.65), which represents a simple condensation reaction between 2,4-dihydroxybenzaldehyde or pyridoxal and aminoguanidine.HCl, was performed as described in detail elsewhere (11). The formed precipitate was filtered and used after re-crystallisation in ethanol:water (1:1 v/v).

Animals

Adult male Wistar rats with body weight of 254 ± 20g were housed and cared for in accordance with the guidelines of the International Guiding Principles for Biomedical Research Involving Animals of the Council for International Organizations of Medical Sciences (CIOMS 1983), which concurred the principles of respect for life. Rats were allowed a standard laboratory pellet chow and free access to water.

Research design

Diabetes was provoked by a single intravenous injection of 45 $mg.kg^{-1}$ b.wt. streptozotocin (dissolved in 0.1 $mol.l^{-1}$ citrate buffer, pH 4.5), whereas control animals received an equal amount of the vehicle (citrate buffer). Beginning with the 2nd day after streptozotocin administration, rats were given daily sub-therapeutic doses of insulin (Interdep, 6 $U.kg^{-1}$, s.c.). Development of the disease was confirmed by two compounding methods: (i) estimating blood glucose levels on the 8th day, and (ii) monitoring glucosuria every 2-3 days (Diabur Test 5000, Roche, Mannheim). Only the animals with blood glucose levels exceeding 15 $mmol.l^{-1}$ were selected for further part of the study. Resorcylidene aminoguanidine was administered subcutaneously at a dose of 4 or 8 mg/kg b.wt. 1st week daily, 2nd week every two days, and from 3rd week every three days. The experiment was terminated after 63 days (9 weeks) i.e., at the stage when diabetic cardiomyopathy was already fully developed, but the hearts had not failed yet (6, 8, 16). Body weight, plasma glucose, plasma total cholesterol, and whole blood glycohaemoglobin were examined three times in all groups: on the same day prior to streptozotocin administration, on the 8th day, and on the last day of the experiment, and estimated using the methods of Tinder (17), Watson (18) and Burrin et al. (19), respectively. On the 63rd day after induction of diabetes all the experimental animals were sacrificed, their hearts removed and subjected to further investigation using the relevant biochemical and biophysical methods, as described below.

Isolation of heart sarcolemmal membranes

Rats were divided into five groups: untreated controls (n = 20, C) and diabetics (n = 20, D) and resorcylidene aminoguanidine-treated controls (n = 18, C + RAG) and diabetics (n = 18, D + RAG). RAG was administered at a dose of 8 mg/kg b.wt. (controls) and 4 or 8 mg/kg b.wt. (diabetic). The isolation of sarcolemmal membranes (SL) was performed according to the method of hypotonic shock as described by Vrbjar et al. (20). The final fraction of isolated SL was resuspended in 10 $mmol.l^{-1}$ Tris-HCL, pH 7.4 and adjusted to the protein concentration of 100 $\mu g.ml^{-1}$. The isolated SL membranes were solubilised in 0.33% polyoxyethylene ether prior to being subjected to the spectrophotometric determination of fructosamine according to Johnson et al. (21). Fructosamine content in the isolated SL membranes was expressed as the absorbance read at 530 nm per mg total sample protein determined according to Lowry et al. (22).

Measurements of SL membrane fluidity

For labelling, the stock DPH solution (5×10^{-4} mol/l in acetone) was freshly diluted 1:250 in in 10 $mmol.l^{-1}$ Tris-HCl (pH 7.4) and vigorously stirred for 30 minutes to remove acetone (23). One volume of thus prepared working DPH solution was then mixed with one volume of the diluted SL suspension containing 50 µg.ml-1 membrane protein to give the final DPH concentration in labelled samples of 1 $\mu mol.l^{-1}$.

Fluorescence anisotropy measurements were made with a Specord M-40 spectrophotometer (Carl Zeiss, Jena). The samples were excited at 360 nm, and the emission was recorded through a standard fluorescence holder (Carl Zeiss Jena). Dichroic filters were used as the polarisers and analysers in the excitation and emission light paths, respectively. The DPH anisotropy changes were monitored at room temperature (22 °C) for an hour with 5 min intervals. The penetration of DPH

molecules into the membranes followed a simple saturation kinetics. In steady-state, DPH molecules became localised in the central region of the membrane lipid bilayer, and the readings of fluorescence anisotropy did not change any further. The time course of the changes in DPH anisotropy values (r_s) was fitted using the method described by Šikurová et al. (24). To characterise dynamic properties of SL membranes we used two parameters: DPH fluorescence anisotropy (r_s) and the order parameter *S*, which reflect the average orientation of the fluorescence probe, in respect to the average orientation of lipid molecules. For rod-like probe such as DPH, both the parameters can be mutually estimated from each other by using the semi-empirical expression $S^2 = (4/3\ r_s - 0.10) / 0.39$ (25). Values of the order parameter determined from the latter expression are known to be very close to those estimated from time-resolved fluorescence data (26). The value of *S* will be zero if the molecules move without restriction (very rapid rotation in very "fluid" membrane lipid bilayer). If the molecules are constrained to wobble, the value of S approaches 1 (restricted probe rotation in very "rigid" lipid bilayer) (25).

Overload of isolated perfused hearts with external calcium

Inasmuch as the protocol of the isolation of SL membranes excluded any further physiological experiments to be performed on the same hearts, another group of animals was used to investigate the induction of Ca^{2+} paradox. Three subgroups were studied: diabetic animals (*n*=60), diabetic rats treated with RAG at a dose of 4 mg/kg b.wt. (*n*=15); and control animals (*n*=60). Excised hearts were perfused at 37°C in a non-recirculating mode with Krebs-Henseleit solution containing 1.6 mmol.l^{-1} $CaCl_2$ and gassed with a mixture of 95 % O_2 and 5 % CO_2. Following the 15 min stabilising perfusion, to overload the isolated hearts with external calcium, we switched over the rinsing standard Krebs-Henseleit buffer for 3 min with the modified Ca^{2+}-free Krebs-Henseleit solution, containing in addition 0.1 mmol.l^{-1} EDTA. Such a Ca^{2+}-deprivation was followed by the 12 min Ca^{2+}-re-admission by means of perfusion with the original Ca^{2+}-containing buffer. The functional parameters, such as the heart rate, amplitude of electrocardiogram, dp/dt and coronary flow, were monitored during the perfusion, as described in detail elsewhere (7, 16).

Statistical analysis

Results were expressed as means ± standard deviation (SD) or means ± standard error of the mean (SEM). Statistical significance was evaluated using two-way ANOVA and Tukey test for multiple comparisons.

RESULTS

Effect of RAG on metabolic disturbances in diabetic animals

Blood glucose and total cholesterol were significantly elevated in streptozotocin-induced diabetic rats compared to control animals (*Table 1*). Diabetic animals showed significantly enhanced glycohaemoglobin, pointing to the state of chronic hyperglycaemia. Likewise, the content of fructosamine in isolated sarcolemmal membranes, considered as a marker of cardiomyocyte protein glycation, remained significantly higher diabetic animals (*Fig. 1*). Interestingly, neither in diabetic nor in healthy control animals the treatment with RAG affected the concentrations of blood glucose, cholesterol or even

Table 1. Blood biochemical parameters in control (C) and streptozotocin-induced diabetic (D) rats treated with resorcylidene aminoguanidine (RAG)

group	plasma glucose (mmol/l)	total plasma cholesterol (mmol/l)	glycated haemoglobin (% of total Hb)
D	19.0 ± 0.1*	4.07 ± 0.36*	7.35 ± 0.56 *
D + 4 mg RAG	21.0 ± 1.2*	3.76 ± 0.18	8.58 ± 0.68 *
D + 8 mg RAG	20.5 ± 1.3*	3.90 ± 0.20***	7.39 ± 0.37 **
C	5.6 ± 0.1	2.30 ± 0.10	4.36 ± 0.04
C + 8 mg RAG	7.6 ± 0.4#	2.72 ± 0.34	4.24 ± 0.06

Data represent means (SEM for the following numbers of animals: D and C, *n*=20, D+4mg RAG, D+8mg RAG and C+8mg RAG, *n*=18. Significance of differences, estimated by means of Tukey test for multiple comparisons, were:
*p < 0.0001, **p < 0.001, ***p < 0.05 compared to control group; # p < 0.05, compared to untreated animals.

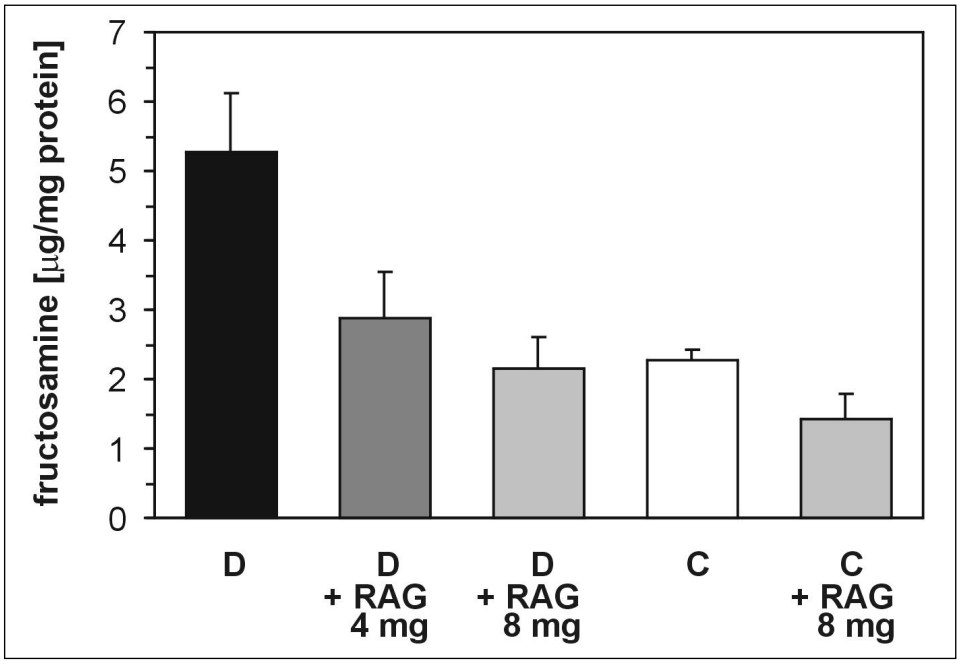


Figure 1. Fructosamine content in sarcolemmal membranes isolated from the hearts of untreated or RAG-treated (4 or 8 mg/kg b.wt.) healthy control (C) and streptozotocin-induced insulin-dependent diabetic rats.
For details see Materials and methods. Results presented as means (SD for the following numbers of animals: D and C, *n*=20, D+4mg RAG, D+8mg RAG and C+8mg RAG, *n*=18. Significance of differences, estimated by means of Tukey test for multiple comparisons, was:
untreated diabetic *vs.* control rats, p < 0.0001; diabetic treated with 4 mg or 8 mg RAG *vs.* diabetic untreated, p < 0.0001; diabetic treated with 4 mg vs. diabetic treated with 8 mg RAG, p < 0.05; control treated with 8 mg RAG vs. control untreated, p < 0.0001.

glycohaemoglobin. However, at both concentrations RAG significantly reduced the formation of fructosamines, reflecting that this anti-glycation agent was able to attenuate he attachment of glucose to proteins of cardiac sarcolemma in a dose-dependent manner not only in diabetic but also in control animals (*Fig. 1*).

Effect of RAG on dynamic parameters of sarcolemmal membranes

The treatment of diabetic rats with RAG significantly fluidised SL membrane lipid bilayer, as demonstrated by the observed decreased values of DPH fluorescence anisotropy. The increased fluidity of membrane lipid bilayer is

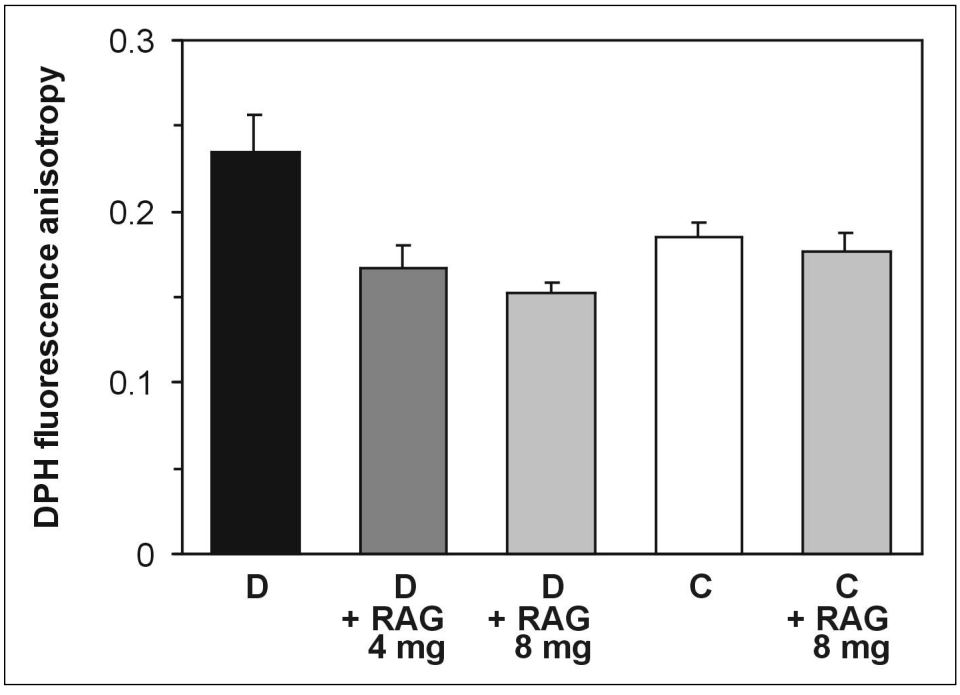


Figure 2. Fluorescence anisotropy of 1,6-diphenylhexatriene-1,3,5 in rat myocardial sarcolemmal membranes isolated from the hearts of untreated or RAG-treated (4 or 8 mg/kg b.wt.) healthy control (C) and streptozotocin-induced insulin-dependent diabetic rats.
For details see Materials and methods. Results presented as means ± SD for the following numbers of animals: D and C, *n*=20, D+4mg RAG, D+8mg RAG and C+8mg RAG, *n*=18. The relevant values of the semi-empirical estimates of the order parameter were:

D	D + 4 RAG	D + 8 RAG	C	C + 8 RAG
0.739 ± 0.050	0.559 ± 0.043	0.513 ± 0.020	0.615 ± 0.026	0.591 ± 0.033

Significance of differences in DPH fluorescence anisotropy and the calculated order parameter (S), estimated by means of Tukey test for multiple comparisons, was:
untreated diabetic *vs.* control, $p < 0.0001$; diabetic treated with 4 mg or 8 mg RAG *vs.* diabetic untreated, $p < 0.0001$.

relevant to the enhanced fluorescence depolarisation, which results in lower anisotropy values and *vice versa*. We showed that both DPH fluorescence anisotropy and the associated order parameter were significantly higher for the group of diabetic animals not treated with RAG ($p < 0.05$) (*Fig. 2*), and this observation clearly pointed to the increased rigidity (relevant to lowered fluidity) of SL membrane lipid layer in diabetic rats compared to control animals. The treatment with RAG of cardiomyocytes from diabetic rats apparently restored DPH fluorescence anisotropy to the values not significantly different from those observed in control untreated rats.

Effect of the overload with external calcium on viability of hearts isolated from control and diabetic animals

To monitor the calcium-resistance of myocardium in RAG-treated and untreated control and diabetic animals and possibly to reason on the impact of the fluidising effect of RAG on SL membranes, we subjected the isolated hearts to the excessive external calcium (termed as "calcium paradox"). Regardless of their treatment with RAG, all the hearts isolated from healthy control animals failed when subjected to the overload with external calcium. Thus, the relative risk that the exposure to RAG affects the survival of hearts isolated from control rats equalled RR=1.0 (95%CI 0.97-1.03, NS), and therefore we concluded that there was no significant association between the failure of hearts overloaded with external calcium and the prior exposure to RAG. Otherwise, fifty of the total 60 diabetic hearts from RAG-untreated animals and 7 of the total 15 diabetic hearts from the rats treated with 4 $mg.kg^{-1}$ RAG survived this heavy calcium overload, thus pointing that the deleterious entry of extracellular calcium was tolerated to much higher extent in the hearts of diabetic animals. The relative risk that the exposure to RAG affects the survival of hearts isolated from diabetic rats equalled RR= 3.20 (95%CI: 1.45-7.05, $p< 0.005$), which means that there is a significant association between the failure of hearts subjected to calcium overload and RAG treatment. Since the chance of the failure of the calcium-overloaded hearts isolated from diabetic rats exposed to RAG was 7/15 (0.533) compared to 10/60 (0.167) in animals non-exposed to RAG, the estimated attributable risk (AR) that RAG increased diabetic heart failure was 0.367, and consequently the calculated proportional attributable risk (PAR) was 68.8%, which means that the exposure of diabetic animals to RAG was responsible for 68.8% of all fatal cases (heart failures) induced by the overload of hearts isolated from diabetic rats to external calcium.

DISCUSSION

Physiological and pharmacological regulation of the function of biological membranes is largely determined by the interactions of membrane proteins and lipids, where the dynamics of phospholipid molecules plays a dominant role. The

overall structural integrity of membrane components, as well as the maintained ordering of the lipid molecules, are the crucial determinants of the optimal fluidity of a lipid bilayer that underlie the proper functioning of membrane transport systems, ion permeability, enzyme activities and receptor responsiveness (27, 28). Using the technique of fluorescence polarisation and the rod-like fluorochrome molecule – 1,6-diphenylhexatriene-1,3,5 (DPH) (23, 25, 26) we have recently documented that in DM, regardless of its type and the presence of late complications, erythrocyte membrane fluidity and its gradient become substantially decreased (14, 15, 29), and these alterations were positively associated with the occurrence of hyperglycaemia, hypercholesterolemia, and elevated glycohaemoglobin content – the apparent hallmarks of diabetes mellitus (30, 31). Evidence accumulated for years also points to the impairments in the membrane functions of heart myocytes, and especially the abnormalities in the cellular Ca^{2+} mobilisation and handling in diabetic heart (7, 32, 33). Our earlier findings (6, 8, 10), showed that in diabetic animals heart myocytes became considerably more resistant to the external overload with Ca^{2+}. It may appear a little paradoxical in respect to a plethora of findings pointing to the DM-induced perturbations in the functioning of ionic transport systems in diabetic cardiomyocytes (6, 7, 34, 35). For a long time such impairments in diabetic cardiomyocytes have been largely attributed to phenomena that involve the formation and action of oxygen free species. The experimental support for the reasoning on such an association was brought by Shao et al., who showed that free radicals might impair the function of myocardium via the inhibition of heart sarcolemmal Na,K-ATPase (36). In our earlier aforementioned studies we have shown that the metabolic state of experimental diabetes and the treatment of diabetic rats with RAG affected the monitored dynamic parameters of cardiac SL membranes in the opposite way. Cardiomyocyte 'membrane rigidification' in diabetic animals was paralleled by the increased non-enzymatic protein glycosylation, as deduced from the reduced DPH fluorescence anisotropy and the enhanced fructosamine formation (6, 8, 10, 14, 15, 29).

Herein we interpret the decreased membrane fluidity in the cardiomyocytes of diabetic rats: (*i*) as resulting from the enhanced glycoxidation, and (*ii*) as associated with some mechanisms of the heart adaptation to permanently present metabolic disorders. The former is in fact a genuine process of detoxication of the unmetabolised glucose, whereas the latter relates to Ca^{2+} overload, originating from the impaired intracellular sequestration of Ca^{2+}, considerably prolonged Ca^{2+} transients, and increased Ca^{2+} influx – the typical phenomena occurring in diabetic cardiac dysfunction (9, 28). To validate whether the decreased SL membrane fluidity may be considered a necessary prerequisite to protect the diabetic heart against Ca^{2+} overload, we used the membrane fluidising agent with a recognised anti-glycation and antioxidant activities – RAG. This agent interfered in diabetic animals with the process of protein glycation (the formation of fructosamine became reduced), and this observation apparently remained in

agreement with our earlier findings reported elsewhere (12, 14, 15, 29, 31). Further, RAG 'fluidised' SL membranes in cardiomyocytes from diabetic rats, however, such an influence was surprisingly associated with elevated susceptibility/vulnerability for heart failure in the RAG-treated diabetic animals. These apparently opposing effects of RAG on the sarcolemmal membrane fluidity require the special comment. Importantly, although it may seem obviously advantageous to pharmacologically prevent excessive glycation, the ongoing glycoxidation and the resulting cardiomyocyte membrane 'rigidisation', such a cure might possibly have a double-edged effect on a diabetic heart. As we stated above, in less fluid cellular membranes encountered in diabetes, the integral proteins embedded into a sarcolemmal membrane lipid bilayer are subjected to a spatial constraint to much higher extent. The restricted mobility of sarcolemmal membrane proteins might be probably due also to their 'clustering' and/or 'cross linking', as a result of the formation of glycation end products under conditions of diabetic chronic hyperglycaemia. Otherwise, upon the interaction with RAG, the formerly tightly ordered phospholipid molecules in cardiomyocyte membrane lipid bilayer might get more spatial freedom, simply because the RAG molecules penetrating sarcolemma lipid bilayer increase the specific volume of the acyl chains in naturally occurring membrane phospholipids. Thus, in the presence of RAG the integral membrane proteins of cardiac sarcolemma acquire some "liberation" in their rotations and/or displacements, and the overall structure of lipid bilayer – initially highly ordered – might become more loosely organised – the phenomenon reflected as diminished DPH fluorescence anisotropy. However, the 'RAG-restored' natural membrane fluidity of diabetic cardiomyocytes evidently made the hearts from diabetic rats 'oversensitive' to the overload with external calcium. Our results point that the overall metabolic disturbances encountered in streptozotocin-diabetic rats seem to make diabetic myocardium more resistant to heavy overload with external calcium, which normal non-diabetic hearts simply cannot stand. Thus, we argue that the DM-induced increase in sarcolemmal membrane rigidity does not merely represent a pathological phenomenon. We suggest that it might be regarded a kind of pathophysiological adaptation to prevent diabetic heart against the entry of excessive calcium, as clearly manifested by a considerably high survival of diabetic hearts subjected to calcium paradox. Accordingly, the reversal of the process of membrane lipid bilayer 'rigidification' by RAG and the restoration of 'circum-natural' physiological membrane fluidity, made diabetic hearts less resistant to calcium and correspondingly depressed their survival under conditions of calcium paradox. Overall, we hypothesise that more rigid SL membranes in diabetic animals might be much less permeable to calcium at the excessive ionic concentrations.

To sum up our observations and hypotheses we need to keep in mind that membrane lipid fluidity directly extrapolates on the effectiveness of a transmembrane passive ionic transport – more fluid membrane lipid bilayer

means faster passive transport of ions inside the cell, and *vice versa*. There are of course further consequences – beyond the objectives of the present study – of the above relationship between the state of membrane fluidity and a facilitation of transmembrane ionic transport. Obviously, we might say that less distorted ionic balance in cardiomyocytes with more rigid and less permeable membranes at the conditions of calcium paradox would mean lesser demand for sarcolemmal membrane ATPases to hold fully active and efficient, and if so, we might expect to observe the reduced ATPases activities in diabetic cardiomyocytes under conditions of calcium paradox. At present, we do not have any brief arguments at hand to support all the above hypotheses or even to show that calcium transport toward the inside of myocardium may be restricted in diabetes upon some situations, like the excessive external calcium gradient. However, our preliminary findings and forthcoming interpretation(s) of our present results in respect of a pathophysiological adaptation of diabetic heart certainly encourage further studies.

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