Cardiovascular Conundra Series *Series Editor:* Karl T. Weber

Subcellular remodeling and heart dysfunction in chronic diabetes

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Received 19 December 1997; accepted 8 June 1998

Abstract

Heart dysfunction in chronic diabetes has been observed to be associated with depressed myofibrillar adenosine triphosphatase activities as well as abnormalities in the sarcoplasmic reticular and sarcolemmal calcium transport processes. The evidence has been presented to show that alterations in the expression of myosin isozymes and regulatory proteins as well as myosin phosphorylation contribute to the development of myofibrillar remodeling in the diabetic heart. Defects in sarcoplasmic reticular and sarcolemmal calcium transport appear to be due to the accumulation of lipid metabolites in the membrane. Different agents, such as calcium-antagonists, beta-adrenoceptor blockers, angiotensin converting enzyme inhibitors, metabolic interventions and antioxidants, have been reported to exert beneficial effects in preventing subcellular remodeling and cardiac dysfunction in chronic diabetes. Clinical and experimental investigations have suggested that increased sympathetic activity, activated cardiac renin–angiotensin system, myocardial ischemia/functional hypoxia and elevated levels of glucose for a prolonged period, due to insulin deficiency, result in oxidative stress. It is proposed that oxidative stress associated with a deficit in the status of the antioxidant defense system may play a critical role in subcellular remodeling, calcium-handling abnormalities and subsequent diabetic cardiomyopathy.

Keywords: Diabetic cardiomyopathy; Myofibrils; Troponin–tropomyosin; Sarcoplasmic reticulum; Sarcolemma; Antioxidants

1. Introduction

In view of the fact that atherosclerosis is commonly seen in diabetic patients, relatively little attention has been paid to studying the problems associated with cardiac muscle during the development of diabetes [1,2]. The possibility of diabetic cardiomyopathy was suggested when diabetic patients at preclinical stages were found to exhibit a shortened left ventricular ejection time, a longer pre-ejection period and an elevated end-diastolic pressure [3]. Such defects in heart function in diabetic patients in the absence of coronary artery disease were considered to be due to increased ventricular wall stiffness, reduced cardiac contractility and a longer isovolumic relaxation [4]. On the other hand, diabetic patients with congestive heart failure showed myocardial hypertrophy and the presence of extensive myocytolytic changes, with replacement fibrosis as well as interstitial and perivascular fibrosis in the heart [5,6]. While chronic diabetes induced by streptozotocin in rats has been shown to be associated with heart dysfunction, including reduced heart rate, depressed peak ventricular pressure as well as depressed rates of contraction and relaxation in the left ventricle [7–12], the combination of diabetes with hypertension in rats has been demonstrated to result in congestive heart failure [5,13]. It is also well known that diabetes is a risk factor for ischemic heart disease; however, it is not clear whether this is due to diabetes-induced microangiopathy [1] or to some other pathogenetic defect associated with insulin-deficiency. Recently, several reviews and articles [14–23] have been devoted to highlighting the underlying basis of diabetes-induced changes in cardiovascular function and metabolism. In this article, we would like to present evidence that heart dysfunction in chronic diabetes is mainly due to subcellular abnormalities in the myocardium. Although an attempt has been made to discuss the mechanisms of this complex problem and to identify the possible cause of

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Time for primary review 31 days.

0008-6363/98/$ – see front matter
PII: S0008-6363(98)00186-2

diabetic cardiomyopathy, it is emphasized that some of the statements in this review represent views of the authors for the purpose of stimulating research in this field.

2. Subcellular remodeling

Animal studies have revealed that heart dysfunction in chronic diabetes is associated with decreased activities of the Ca^{2+} ATPase of cardiac myofibrils, actomyosin and myosin [8,10,12,24,25]. Since the ability of diabetic heart to generate contractile force is dependent upon the magnitude of myofibrillar ATPase activation by Ca^{2+}, the depressed ATPase activities of contractile proteins can be seen to play an important role in the development of heart dysfunction due to diabetes. It has been identified that the depressed ATPase activity of myofibrils is due to alterations in myosin isozyme composition and regulatory proteins as well as to the phosphorylation of regulatory proteins in the diabetic heart [24–27]. It should be pointed out that diabetes is associated with a shift in myosin isozyme content from V_1 to V_3 in the heart [28–30]. The analysis of α- and β-myosin heavy chain (MHC) mRNA and protein expression indicated the predominance of α-MHC and β-MHC in control and diabetic hearts, respectively. Different Ca^{2+}-antagonists, metabolic interventions and exercise were found to improve the contractile function of the diabetic heart, increase myosin Ca^{2+}–ATPase activity and prevent the shift in myosin isozymes [1,7,28,31]. It should be mentioned that the human heart primarily expresses V_3 or the β-isoform of myosin [32,33] and, thus, the shift of myosin isozymes observed in diabetic animal hearts may not be of physiological significance in the adaptation of human heart in diabetes. On the other hand, since the phosphorylation of myosin light chain (MLC) by myosin light chain kinase (MLCK) has been shown to play a modulatory role in the generation of contractile force in vertebrate striated muscle, MLC phosphorylation in cardiac muscle fibers showed a leftward shift in the force–*p*Ca relationship and decreased cooperativity [34]. Recently, Liu et al. [26] have reported that the protein contents of MLC and MLCK as well as MLC phosphorylation were decreased significantly in the diabetic rat heart homogenate; these changes were partially reversible upon treatment with insulin.

The troponin–tropomyosin (TnTm) complex is made up of three troponin (Tn) subunits (TnC, the Ca^{2+} binding unit; TnI, the ATPase inhibitory unit and TnT, the tropomyosin binding unit) and tropomyosin. Earlier investigations have suggested that phosphorylation of TnI and TnT by protein kinase C (PKC) results in a decrease in the actomyosin ATPase activity and a decrease in the actin–myosin interaction. Phosphorylation of TnI and TnT by protein kinase A (PKA) has been associated with a reduced sensitivity of the myofibrillar Mg^{2+}–ATPase to Ca^{2+}. Thus, changes in the regulatory effects of the TnTm complex on actin and myosin can be seen to explain the depressed myofibrillar ATPase activities in the diabetic heart. In fact, the decreased actomyosin ATPase activity in the hearts of diabetic animals was partially reversed when myosin from diabetic rats interacted with the TnTm protein complex isolated from control hearts [35]. Although no significant changes in protein content and gene expression of TnI were observed in the right and left ventricles from the diabetic rats, the phosphorylation of TnI was higher in the diabetic hearts [27]. Other studies have suggested that the increased phosphorylation of TnI in the diabetic hearts is a result of changes in the subcellular distribution of PKC isozymes [36]. Some studies focused on the relationship between the cardiac TnT isoforms and the force–*p*Ca characteristics of the diabetic heart and have observed a significant shift from TnT_1 to TnT_2 and TnT_3 [37]. These findings have raised the possibility that changes in the Ca^{2+}-sensitivity of myofibrils in the diabetic myocardium are coupled with TnT alterations. Thus, it is evident that both contractile and regulatory proteins are remodeled in diabetic cardiomyopathy and that these changes are reflected by decreased myofibrillar ATPase activity and heart dysfunction.

A large body of evidence has accumulated to suggest the occurrence of remodeling of both sarcoplasmic reticulum and sarcolemmal membranes in the heart during the development of chronic diabetes [1,38,39]. Previous studies have shown a depression in the sarcoplasmic reticular Ca^{2+}-pump activity [9,40], which is considered to account for the inability of the diabetic heart to relax fully. However, the molecular mechanism for the sarcoplasmic reticular Ca^{2+}-pump depression are not clear because conflicting results concerning changes in mRNA level (gene expression) for the Ca^{2+}-pump protein have been reported in the diabetic heart [41–44]. The occurrence of Ca^{2+}-handling abnormalities in the cardiac cell has also been attributed to defects in the sarcolemmal Na^+–Ca^{2+} exchange and Ca^{2+}-pump activities in diabetic hearts [45,46]. Sarcolemmal Na^+–K^+ ATPase and Ca^{2+}-binding activities were decreased [47,48], whereas sarcolemmal Ca^{2+}/Mg^{2+} ecto-ATPase activities were increased [49,50] in diabetic cardiomyopathy. On the basis of differences in the control and diabetic hearts with respect to their electrophysiological and mechanical behavior in the presence of different concentrations of Ca^{2+}, abnormalities for Ca^{2+}-handling in diabetic hearts were also indicated [51]. Although the basal levels of intracellular free Ca^{2+}, as well as KCl- and ATP-induced increases in the intracellular concentration of free Ca^{2+}, in diabetic cardiomyocytes were not different from control preparations [52], the results regarding this aspect are controversial [19,20]. Such differences in results from various studies may be due to differences in the techniques employed for the measurement of the intracellular concentration of free Ca^{2+} and/or the intensity as well as the duration of diabetes. Nonetheless, abnormalities in Ca^{2+}-handling by diabetic myocar-

dium were evident from observations showing attenuated responses of diabetic cardiomyocytes to catecholamines, 8-bromo-cAMP and phosphatidic acid with respect to increasing the intracellular concentration of free Ca^{2+} [19,52]. In addition, the significance of defects in sarcoplasmic reticular and sarcolemmal membranes are evident from the depressed responsiveness of diabetic heart to rapid cooling, caffeine and other interventions that are known to affect these sites [19,20].

It should be pointed out that the changes observed in the cell membrane of the diabetic heart would favor the occurrence of intracellular Ca^{2+} overload in cardiomyocytes. It is emphasized that the intracellular Ca^{2+} overload referred to here should not be confused with the intracellular concentration of free Ca^{2+}, which may not change because free Ca^{2+} in the cytoplasm may become accumulated in the intracellular organelles of cardiomyocytes. In fact, elevated levels of myocardial Na^{+} and Ca^{2+} content have been reported in diabetes [53,54] and intracellular Ca^{2+}-overload has been shown to result in functional abnormalities in the heart [55,56]. Furthermore, verapamil, a well known Ca^{2+}-antagonist, was found to produce beneficial effects in diabetic cardiomyopathy because it attenuated changes in heart function, ultrastructure, subcellular activities and Ca^{2+} content in diabetic animals [28,54]. Verapamil has also been shown to prevent the increase in cardiac PKC activity in diabetic rats [57]. Although depressions in mitochondrial Ca^{2+}-uptake, stage 3 respiration, respiratory control index and Mg^{2+}-ATPase activities were also observed in diabetic heart [58], these alterations in the mitochondrial membrane were seen subsequent to changes in both the sarcolemma and sarcoplasmic reticulum. Such observations, however, do not rule out changes in mitochondrial function due to the occurrence of intracellular Ca^{2+} overload in diabetic cardiomyocytes. Thus, from the foregoing discussion, it is evident that remodeling of both sarcolemmal and sarcoplasmic reticular membranes may result in Ca^{2+}-handling abnormalities in cardiomyocytes and subsequent heart dysfunction during the development of diabetic cardiomyopathy.

3. Mechanisms of subcellular abnormalities

3.1. Insulin deficiency

Several studies have indicated that the basal as well as insulin-stimulated glucose uptake in the diabetic heart is reduced due to either insulin deficiency or insulin resistance [59–61]. The reduced levels of mRNA and protein of the glucose transporter, GLUT4, have been observed in diabetic hearts from experimental animals and patients [62–65]. It is also clear that severe insulin deficiency results in large decreases in the activation of cardiac glycogen synthase and phosphatase activity [66]; a 20–25% decrease in the protein phosphatase 1 catalytic subunit has been reported [67]. The hypersensitivity of phosphorylase activation in hearts from diabetic rats is well documented [66,68,69]; this abnormality was corrected by perfusion of diabetic hearts with low concentrations of calcium or reversed by adrenalectomizing the diabetic rats [69]. Since the regulation of calcium transport in the sarcoplasmic reticulum involves protein phosphatases [70], the diabetes-related hypersensitivity for the activation of phosphorylase can be explained by an increase in intracellular calcium or by a decrease in phosphorylase phosphatase. The role of changes in myocardial metabolism in the diabetic heart is further evident from the fact that different metabolic interventions, such as carnitine and etomoxir, which are known to depress the oxidation of free fatty acids and promote glucose utilization, were found to improve cardiac function in chronic diabetes [31,71]. Thus, a shift in myocardial metabolism resulting in an excessive use of free fatty acids and a marked reduction in the utilization of plasma glucose can be seen to play a critical role in the development of diabetic cardiomyopathy. In fact, accumulation of long chain fatty acids in the sarcoplasmic reticulum has been shown to alter the function of this membrane system in the diabetic heart [72] and a similar mechanism can also be seen to explain the defects in the sarcolemmal membrane in chronic diabetes. Alterations in the sarcoplasmic reticular and sarcolemmal membranes in diabetic heart have also been shown to be due to changes in the membrane phosphatidylethanolamine N-methylation [73,74]. Thus, it appears that insulin deficiency promotes alterations in the lipid composition of cardiac membranes and these may then be associated with remodeling of both sarcoplasmic reticulum and sarcolemma during the development of diabetic cardiomyopathy.

3.2. Sympathetic nervous system

The involvement of increased sympathetic activity in diabetes is evident from observations showing the beneficial effects of β-adrenoceptor blockers in improving organ dysfunction [1]. Although an excessive amount of catecholamines are circulating in the body due to activation of the sympathetic nervous system at early stages of diabetes [75,76], there is an ample body of evidence showing depressed responses of diabetic heart to these hormones [77,78]. The attenuated responses of the diabetic myocardium to catecholamine have been shown to be due to a decreased number of β-adrenergic receptors and depressed epinephrine-stimulated adenylyl cyclase activities in diabetic cardiomyopathy [78,79]. The reduced number of adrenergic receptors and uncoupling of the adenylyl cyclase system in the myocardium of diabetic animals have also been observed by other investigators [80–82]. Such alterations in the diabetic heart have been attributed to a defect in the cell membrane as a consequence of increased

oxidation of catecholamines, which produces toxic substances such as oxyradicals and adrenochrome [1].

3.3. Renin–angiotensin system

The role of the renin–angiotensin system in the development of diabetic cardiomyopathy is apparent from the fact that different angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor blockers were observed to prevent diabetes-induced heart dysfunction, myocardial perfusion defects, alterations in sympathetic nerves, myocardial as well as interstitial fibrosis and a decline in the levels of glucose transporter in the myocardium [83–87]. Since the plasma renin–angiotensin system was either down-regulated or unaltered in diabetes [88–90], activation of the cardiac renin–angiotensin system in diabetes is more likely. This view is based on observations that the density and mRNA levels of angiotensin II receptors in the diabetes heart are increased [91–93]. Whether this cardiac renin–angiotensin system is involved directly or indirectly in inducing remodeling of the heart during diabetes is not clear at present, however, activation of the cardiac renin–angiotensin system can be seen to result in oxidative stress and this may lead to the development of diabetic cardiomyopathy. This concept is supported by observations that ACE inhibitors were found to increase both the enzymatic and nonenzymatic antioxidant defense mechanisms in the heart and other tissues [94]. Furthermore, ACE inhibitors were found to reduce the ischemia–reperfusion-induced formation of oxidized glutathione, accumulation of Ca^{2+} in mitochondria and the release of norepinephrine from the heart [95]. Thus, activation of the renin–angiotensin system can be considered to promote the formation of oxyradicals due to changes in mitochondrial function and the release of norepinephrine in addition to augmenting the activity of oxyradicals as a consequence of depletion of the tissue's antioxidants.

3.4. Thyroid deficiency

Diabetes mellitus is associated with a low thyroid state, as characterized by low serum thyroid hormone values in patients and animals [96–98]. The treatment of diabetic rats with pharmacological doses of T3 (triiodothyronine) or T4 (thyroxine) effectively reversed the decrease in myosin ATPase activity, without affecting the depression in cardiac function [29,31,99]. The enhanced positive inotropic response to α-adrenergic stimulation in isolated atria of diabetic rats was also prevented by T3 treatment [100]. In addition, it has been reported that the regulation of cardiac β-MHC expression by insulin is a complex mechanism involving the interaction of insulin with subcellular factors that have an impact on the specific action of T3; this interaction may be disrupted in the absence of sufficient thyroid hormone [101]. Since alterations in sarcoplasmic reticular Ca^{2+}-transport as well as in cardiac function, unlike the observed changes in contractile proteins, were not modified by the treatment of diabetic animals with thyroid hormones [9,99], it appears that diabetes-induced remodeling of cardiac membranes, unlike myofibrillar proteins, may not involve thyroid hormone deficiency in chronic diabetes.

3.5. Myocardial ischemia

A rapid rate of ventricular failure during ischemia has been observed in diabetic rat hearts [102]. Abnormalities of the coronary microcirculation, distinct from large-vessel atherosclerosis, have been reported in clinical and experimental diabetes mellitus. In this regard, morphological studies have indicated defects in the resistance vessels in diabetic patients and animals as well as functional abnormalities of the coronary circulation of diabetic animals [103,104]. Coronary vasodilation in response to norepinephrine, calcium and paced tachycardia was impaired in the isolated diabetic rat hearts [105]. The resting coronary blood flow in diabetic lambs was reduced over a wide range of aortic pressures in comparison with the control animals [106]. The peak reactive hyperemic response after a brief coronary occlusion as well as due to adenosine was attenuated in anesthetized diabetic dogs [107]. Basal production of thromboxane by coronary artery rings was increased whereas production of prostacyclin by coronary rings during hypoxia and α-adrenergic stimulation was impaired in diabetic rats [108]. Nitenberg et al. [109] reported a reduced maximal coronary blood flow reserve and impaired endothelial-dependent epicardial coronary vasodilation in diabetic patients. Nahser et al. [110] observed a reduction in the maximal coronary vasodilation as well as impaired regulation of coronary flow in response to submaximal increases in myocardial demand in patients with diabetes mellitus. These microvascular abnormalities suggest the occurrence of myocardial ischemia in the absence of coronary atherosclerosis in diabetic patients and animals. Thus, microangiopathy can be seen to result in myocardial ischemia and/or functional hypoxia, which may be one of the crucial factors for the occurrence of oxidative stress and remodeling of the subcellular organelles in the diabetic heart.

3.6. Oxidative stress

Clinical and experimental evidence has suggested that free-radical-mediated oxidative processes are involved in the pathogenesis of diabetic complications [111–115]. An increase in the production of free radicals can result from the hyperglycemia-induced enhancement in glucose autoxidation, protein glycation and subsequent oxidative degradation of glycated proteins [116–118]. Since cellular defense mechanisms, such as antioxidants and antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase), play a fundamental role in protecting the cell

against reactive free radical and other oxidant species [119], it is possible that a loss of these factors will promote the occurrence of oxidative stress during the development of diabetic cardiomyopathy. However, increases in superoxide dismutase, glutathione peroxidase and catalase activities have been observed in diabetic rat heart [120–122]. Although increased levels of tissue antioxidant may serve as an adaptive mechanism during the early stages of diabetes, this antioxidant reserve may get depleted with time and the balance may be tilted towards oxidative stress and subsequent heart dysfunction. This view is consistent with some studies indicating that oxidative stress may play a role in the development of perivascular fibrosis and severe changes of the autonomic nerves and endothelial system in the myocardium [123]. Not only the status of the tissue antioxidant level determines the degree of oxidative stress and organ function, the impairment of endothelium may also promote oxidative stress activity. Although a direct relationship between endothelial changes and the occurrence of diabetic cardiomyopathy has not been examined thus far, a defective endothelium has been shown to produce an imbalance, due to reduced production of nitric oxide and increased formation of oxyradicals, and is thus considered to promote the risk of coronary artery disease [114]. On the other hand, an excessive amount of nitric oxide produced due to endothelial dysfunction and superoxide radicals formed as a consequence of increased autoxidation of glucose and protein glycation [116–118,123] can also be seen to react with each other to result in the formation of a strong oxidant, peroxynitrite and, thus, promote the occurrence of oxidative stress in diabetes. Nonetheless, the role of oxidative stress in diabetes-associated complications in different organs, including diabetic cardiomyopathy, is evident from observations that the treatment of diabetes with vitamin E and other antioxidants has been shown to increase the tissue's antioxidant levels, improve the action of insulin, promote endothelial function, prevent oxidation of serum lipids, suppress protein glycation and reduce the oxidative load [124–130]. A direct relationship between oxidative stress and the development of cardiomyopathy in chronic diabetes, however, remains to be established.

4. Concluding remarks

Since diabetes is associated with increased levels of circulating catecholamines as well as myocardial ischemia/hypoxia, which are known to promote the formation of oxyradicals and oxidants, it is likely that oxidative stress may play a central role in the development of subcellular remodeling and subsequent heart dysfunction in chronic diabetes. An initial increase in the level of tissue antioxidants can be seen to maintain organ function and, thus, depletion of tissue antioxidants would favor the occurrence of oxidative stress. An insulin-deficiency-associated metabolic shift as well as an increase in plasma glucose levels would also result in the increased formation of oxyradicals, due to increased autoxidation of glucose and dramatic alterations in mitochondrial function. Activation of renin–angiotensin, which results in the depletion of tissue antioxidant, as well as the impaired function of endothelium, may also produce conditions favoring oxidative stress in diabetes. Accordingly, a scheme showing the involvement of oxidative stress in the development of diabetic cardiomyopathy is shown in Fig. 1. Support for

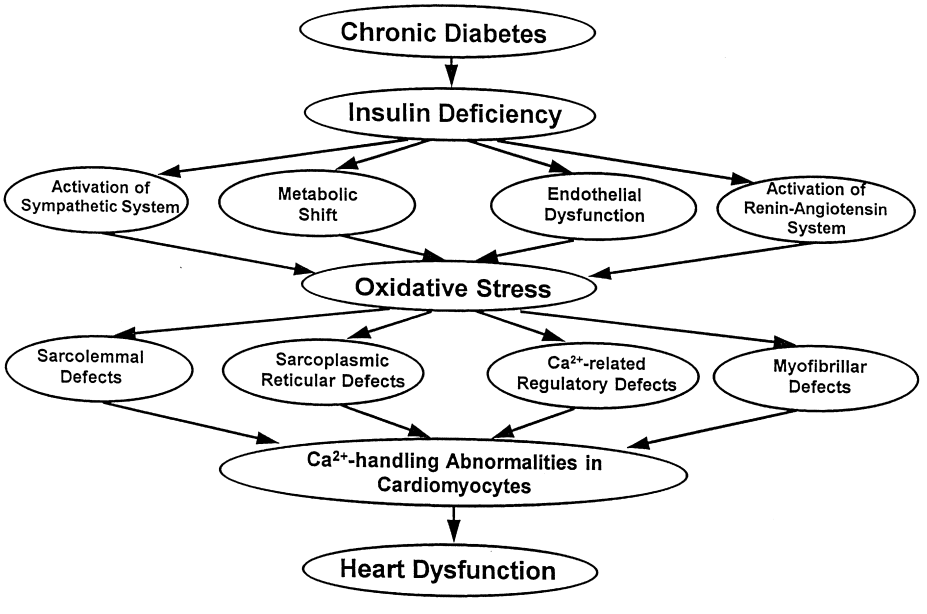

Fig. 1. Proposed mechanisms for the remodeling of subcellular organelles involving oxidative stress as a focal point for heart dysfunction during the development of diabetic cardiomyopathy.

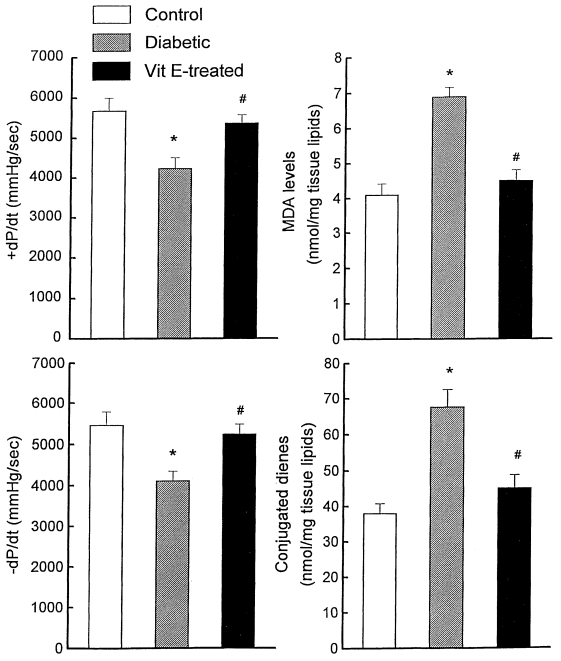

Fig. 2. Alterations in the left ventricular rate of contraction (+dP/dt) and rate of relaxation (−dP/dt) as well as malondialdehyde and conjugated dienes contents in diabetic rats with or without vitamin E treatment. Diabetes in rats was induced by an injection of streptozotocin (60 mg/kg, i.v.) and the animals were assessed hemodynamically eight weeks later by methods given elsewhere [9,54]. At the end of the hemodynamic assessment, the left ventricle was processed for biochemical analysis. Control rats received an injection of citrate buffer. Treatment of rats with vitamin E (25 mg/kg/day, i.p.) was started 24 h after inducing diabetes. Each value is a mean±SE of six experiments. *P<0.05 in comparison to control; #P<0.05 in comparison to the untreated diabetic group.

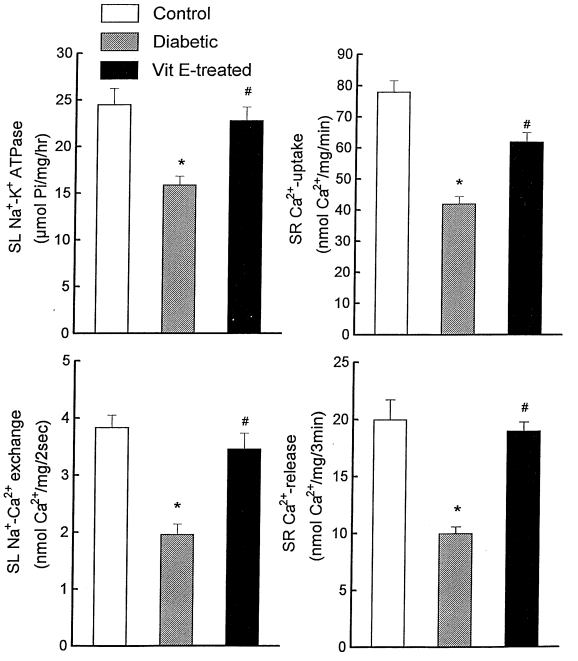

Fig. 3. Alterations in the left ventricle sarcolemmal (SL) Na^+–K^+ ATPase and Na^+–Ca^{2+} exchange activities as well as sarcoplasmic reticular (SR) Ca^{2+}-uptake and Ca^{2+}-release activities in diabetic rats with or without vitamin E treatment. The methods for inducing diabetes and vitamin E treatment were the same as described in the legend for Fig. 2, whereas the procedures for the assessment of SL and SR functions are same as given elsewhere [12]. Each value is a mean±SE of six experiments. *P<0.05 in comparison to control; #P<0.05 in comparison to the diabetic group.

this hypothesis is evident from our experiments in which the treatment of streptozotocin-induced diabetes in rats with vitamin E was observed to prevent the attenuated rate of contraction (+dP/dt) and rate of relaxation (−dP/dt) as well as augmented levels of malondialdehyde and conjugated dienes in the diabetic heart (Fig. 2). Furthermore, treatment of diabetic animals with vitamin E was found to prevent depressions in cardiac myofibrillar Ca^{2+}–ATPase activity (Table 1), sarcolemmal Na^+–K^+ ATPase and Na^+–Ca^{2+} exchange activities as well as sarcoplasmic reticular Ca^{2+}-uptake and Ca^{2+}-release activities in chronic diabetes (Fig. 3). Since we did not measure the free radical species in diabetic animals with or without vitamin E treatment, the interpretation of the beneficial effects of vitamin E in terms of its antioxidant activity should be made with some caution. Although some caution

Table 1
General characteristics and cardiac myofibrillar ATPase activities in diabetic rats with or without vitamin E treatment

	Control	Diabetic	Diabetic+vitamin E
Body weight (g)	495±16	301±17[a]	315±21[a]
Ventricular weight (g)	1068±124	826±41[a]	842±33[a]
Plasma glucose (mg/dl)	151±7.6	487±9.2[a]	478±8.8[a]
Plasma insulin (μU/ml)	28±2.4	11±0.7[a]	12±0.9[a]
Myofibrillar Ca^{2+}-stimulated ATPase activity (μmol P_i/mg/h)	11.6±0.9	5.8±0.5[a]	8.4±0.4[a]
Myofibrillar Mg^{2+} ATPase activity (μmol P_i/mg/h)	3.8±0.2	3.7±0.2	3.9±0.3

The methods for inducing diabetes and the vitamin E treatment were the same as described in the legend for Fig. 2, whereas the procedures for the isolation and measurement of myofibrillar ATPase are the same as given elsewhere [25].
Each value is a mean±SE of six experiments.
[a]P<0.05 in comparison to control.

should also be exercised while extrapolating the animal data to patients with diabetes, this study suggests a need for clinical trials with antioxidants in the diabetic population.

Acknowledgements

The research work reported in this article was supported by a grant from the Medical Research Council of Canada (MRC Group in Experimental Cardiology).

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