Changes in Cardiac Contractility in IDDM and NIDDM Diabetic Rats

I. BÁNYÁSZ, I. KALAPOS*, S. KELLEMEN and T. KOVÁCS

Department of Physiology, University Medical School of Debrecen, Debrecen, Hungary

Abstract. Differences in myocardial contractility were studied in type I (insulin-dependent, IDDM) and type II (non-insulin dependent, NIDDM) diabetic rats. Using the streptozotocin-induced diabetes as an experimental model, the contractile properties of left ventricular myocardium of IDDM and NIDDM animals were compared to similar parameters of their age-matched controls. Contraction force was analyzed as a function of the pacing frequency. Paired-pulse stimulation and catecholamine treatment were applied to compare the inotropic responses obtained in the two types of diabetes. Diabetic and control preparations developed equal peak tension at each driving frequency upon the application of paired-pulse stimulation with fixed interpulse interval. The interpulse interval dependence of paired pulse induced inotropy was altered and the velocity of contraction and relaxation decreased in IDDM but not in NIDDM muscles. Sensitivity to isoproterenol and norepinephrine was decreased in both types of diabetes; however, the isoproterenol resistance of old diabetic animals was attributable to age rather than to the diabetic state. The results indicate that alterations in the contractile parameters and catecholamine sensitivity in IDDM differ from those observed in NIDDM form of diabetes mellitus.

Key words: Diabetes — Stimulated myocardium — Rat — Contractility — Catecholamine

Introduction

Mechanical dysfunction of diabetic myocardium has been studied extensively during the last decade. While the altered cardiac function in IDDM has been char-

Correspondence to: Tamas Banyasz, M.D., Ph.D., Department of Physiology, University Medical School of Debrecen, H-4012 Debrecen, P.O. Box 22, Hungary. Phone: (36)-52-416-631, Fax: (36)-52-132-289, E-mail: TOMO@PHYS.DOTE.HU

*Present address: Department of 1st Internal Medicine, University Medical School of Debrecen, Debrecen, Hungary

acterized in detail our knowledge is relatively poor regarding these alterations in NIDDM. Decreased velocity of both contraction and relaxation were observed in IDDM by several investigators (Rubinstein et al. 1984; Atkins et al. 1985; Heyliger et al. 1986; Rosen et al. 1986; Schaffer et al. 1989; Tanaka et al. 1992; Downing et al. 1993) whereas others reported similar changes in NIDDM as well (Schaffer et al. 1985; Sakai et al. 1992). Unaltered velocity of contraction with or without decreased velocity of relaxation was reported in IDDM but not in NIDDM (Fein et al. 1980; Penpargkul et al. 1980; Verma and McNeil 1994). In IDDM developed ventricular pressure was decreased with or without normal cardiac output (Penpargkul et al. 1980; Rubinstein et al. 1984; Heyliger et al. 1986; Rosen et al. 1986; Schaffer at al. 1989; Tanaka et al. 1992; Verma and McNeil 1994) whereas NIDDM produced no change in the ventricular systolic pressure (Fein et al. 1980) in spite of the decrease in cardiac work observed (Sakai et al. 1992; Schaffer and Wilson 1993). The heterogeneous observations might in part be a result from the perfused heart model used in these experiments since different experimental conditions (i.e. filling pressure, ionic milieu, severity of diabetes etc.) might alter cardiac function too.

In addition, controversial results have been obtained with regard to the magnitude of the developed tension of diabetic myocardium in IDDM. Practically all potential alterations of myocardial force production have been reported. Using ventricular muscle strips, papillary or trabecular muscles of the rat decreased (Fein et al. 1981) or increased (Xiang and McNeil 1991a) as well as unaltered (Fein et al. 1980) contractile force has been reported. Catecholamine induced inotropy has been studied by several authors, however no attempt has been made to compare the absolute values of tension in diabetic and healthy animals (Yu and McNeil 1990; Warner et al. 1991; Xiang and McNeil 1991b). Similarly no data on absolute values of tension are available in NIDDM rats. With respect to other contractile parameters like time to peak tension or half relaxation time researchers again arrive at unanimous conclusions. It is generally accepted that the duration of the contraction curve is prolonged in diabetic animals compared to controls.

The present study was performed to characterize the tension development and its time course during the contractile cycle in healthy and diabetic animals at various states of the disease. We desired to obtain information on the force frequency relationship and the inotropic response of diabetic myocardium. To achieve this five characteristic parameters (peak tension, time to peak tension, maximum velocity of contraction, half relaxation time, and area under the contraction curve) of the contracting myocardium were compared in normal and diabetic (both IDDM and NIDDM) cardiac preparations paced at different driving rates. Inotropic responses were evaluated by applying isoproterenol (ISO) and norepinephrine (NE). Since the effects of both ISO and NE are known to be complex on working cardiac tissue, we induced inotropy using paired pulse stimulation as well. The benefit

of application of paired-pulse protocol is that sarcolemmal receptor functions and subsarcolemmal signalization are not involved in the mechanism of this kind of inotropy. Comparing these pharmacologically induced (catecholamine) and directly induced (paired-pulses) inotropic responses the mechanisms involved in diabetic alterations of cardiac force generation may be better elucidated.

Materials and Methods

Induction of type I (IDDM) diabetes

Adult, randomly selected Wistar rats of either sex weighing 120–150 g, were injected into tail vein with 65 mg/kg Streptozotocin (STZ), dissolved in citrate buffer. Age matched control animals were injected with the vehicle only. Hyperglycemia was confirmed by blood glucose test using enzymatic techniques. First blood glucose test was performed 48 hours after the STZ treatment, then the tests were repeated weakly. In animals treated with STZ blood glucose levels increased above 10 mmol/l. Experiments were performed following 4 weeks (28 days ±2 days) of the STZ treatment.

Induction of type II (NIDDM) diabetes

Neonatal animals of healthy Wistar parents were subdivided into two groups randomly at age younger than 48 hours. According to the method of Schaffer and his colleagues (Schaffer et al. 1989, Schaffer and Wilson 1993) one group of animals was treated with 90 mg/kg STZ i.p. (controls received the vehicle only), then the pups were given back to their mothers. Animals were used for experiment at the age of 12 months. The diabetic state of the STZ treated animals was followed by blood glucose monitoring and glucose tolerance tests. Glucose load (in dose of 1 g/kg body weight) was performed at the beginning and at the end of the first hour of the glucose tolerance test (double load test). Blood samples were taken at 0, 0.5, 1, 2, 3 and 4 hours after the first glucose load, then glucose concentration was determined enzymatically from the samples.

The animals were fed on standard laboratory diet receiving water and food ad libitum, and were maintained on natural light and dark cycle in a constant temperature environment at 24 °C.

Measurement of contractility

Trabecular muscles were carefully excised from the left ventricle and mounted in a thermoregulated (32 °C) experimental chamber continuously perfused with oxygen saturated Tyrode solution containing (in mmol/l) NaCl 150, KCl 5, $MgCl_2$, 1.5, $CaCl_2$ 2.5, HEPES 5 at pH 7.24. The chamber volume was refreshed 9–11 times per minute. Pairs of parallel platinum electrodes were used for field stimulation

(40 mA/cm^2) pacing the muscle continuously at a constant frequency with 2 ms wide rectangular pulses. Resting length of the muscle was adjusted so as to develop maximal active tension under isometric conditions. The capacitive transducer used in our experiments provided linear voltage response in the range of 0.5–15 mN. The analogous electrical signal was digitized and analyzed on IBM compatible PC using Sigmaplot and Sigmastat scientific softwares (Jandel Corporation, San Rafael, CA, USA). The contractile parameters (developed peak tension (P), time to peak tension (ttP), rising velocity of developed tension (dP/dt), half relaxation time (HRT) and area under the contraction curve ($Integral$)) were calculated and stored for further analysis. Rising rate of the contraction curve was estimated by linear fitting to the 25–75% range of the rising phase, then it was normalized to the peak tension. Apart from the computer used for data analysis, all equipments were developed and manufactured at our department.

A typical experiment was performed as follows. After mounting the preparation in the experimental chamber, a 60 min period of equilibration at a constant pacing frequency of 0.5 Hz was allowed to stabilize the contractile parameters of the muscle. Then the stimulation frequency was set to the desired value and kept constant for further 10 min when the first measurement was made. Depending on the pacing frequency, 10 to 25 consecutive contractions were recorded and the data were stored. When paired-pulse stimulation was applied, 25 to 30 contractions were evoked before recording. During this time, the inotropic response reached its maximum level and the contractile parameters stabilized. In the experiments using catecholamines, the drugs were applied for 10 min in increasing concentrations.

All chemicals were purchased from SIGMA Chemicals (St. Louis, USA). Groups of preparations were compared using ANOVA analysis. Student-Newman-Keuls test was used to determine statistical significance of the changes (Zar 1984). Changes were considered significant when p was less than 0.05. Data are expressed as means ± SEM. A summary of the experimental groups and their interpretations is provided in Table 1. Abbreviations of group names will be consistently used throughout the text. The entire investigation conformed to the guidelines for

Table 1. Groups of experimental animals

Group name	Abbreviation	Interpretation
Young diabetic	YD	4 months old rats with STZ-induced IDDM
Young control	YC	Age-matched control group of YD
Old diabetic	OD	12 months old rats with STZ-induced NIDDM
Old control	OC	Age-matched control group of OD

It should be noted that all animals were adults when used. Terms young and old refer to the different age of these animals (4 and 12 months in the case of young and old groups, respectively). For further details see the text.

the care and use of laboratory animals published by the US National Institutes of Health as well as to the principles outlined in the Helsinki Declaration

Results

Cardinal symptoms of IDDM such as increased blood glucose level polyphagia polydipsia polyuria and loss of weight were observed in rats of the YD group Blood glucose concentration was significantly elevated in the YD group (21 3±1 47 mmol/l comparing to the control value of 5 2 ± 0 35 mmol/l in YC $p < 0\ 001$) In contrast to the YD animals the moderate elevation of blood glucose level was not significant in the OD group However decreased glucose tolerance following the double glucose load revealed the presence of NIDDM in the OD group (Fig 1) Fasting blood glucose concentrations were identical in the OC and OD groups but following the glucose loads the elevated blood glucose level was prolonged in the OD animals

Also parameters of contraction changed markedly in IDDM Since the shape of the contraction curve was altered by paired pulse stimulation itself only twitches evoked by single pulses were examined this way Contraction of the left ventricular myocardium was prolonged in the YD group compared to YC The prolongation developed as early as 2 weeks after the STZ treatment and reached its maximum after week 4 (Fig 2) Time to peak tension (*ttP*) and half relaxation time (*HRT*)

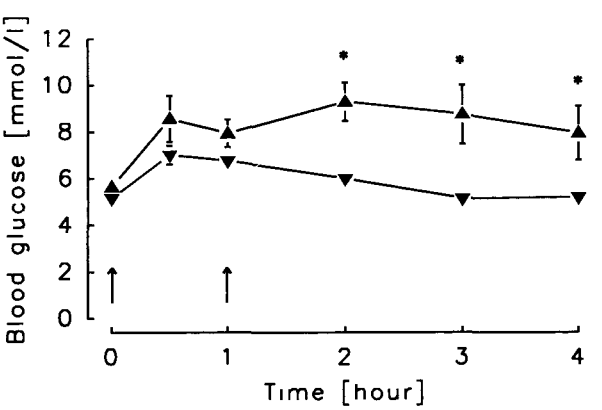

Figure 1. Glucose tolerance test in 12 months old Wistar rats ▼ Old control (OC) ▲ old diabetic (OD) The arrows mark the time of the glucose loads (0 and 60 min) The mean glucose levels in the NIDDM group were significantly higher (asterisks denote $p < 0\ 05$) than the time matching control values measured following the second glucose load Each data point represents mean ± S E M obtained from 27 animals

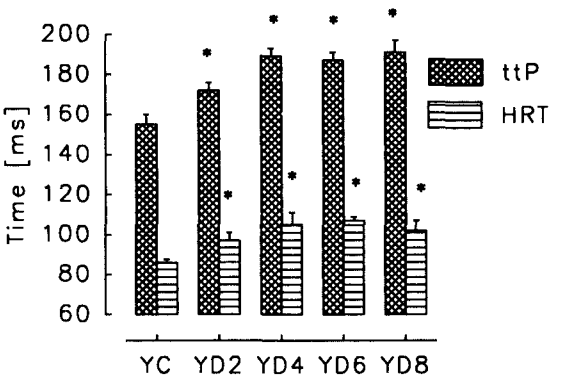

Figure 2. Time to peak tension (*TTP*) and half relaxation time (*HRT*) values of trabecular muscles for control (YC) and diabetic (YD2 – YD8) rat hearts. Measurements in diabetic animals were performed 2 (YD2), 4 (YD4), 6 (YD6) and 8 (YD8) weeks following the induction of diabetes. Data represents mean ± S.E.M. of 10–19 animals. The asterisks denote significant differences from control ($p < 0.05$).

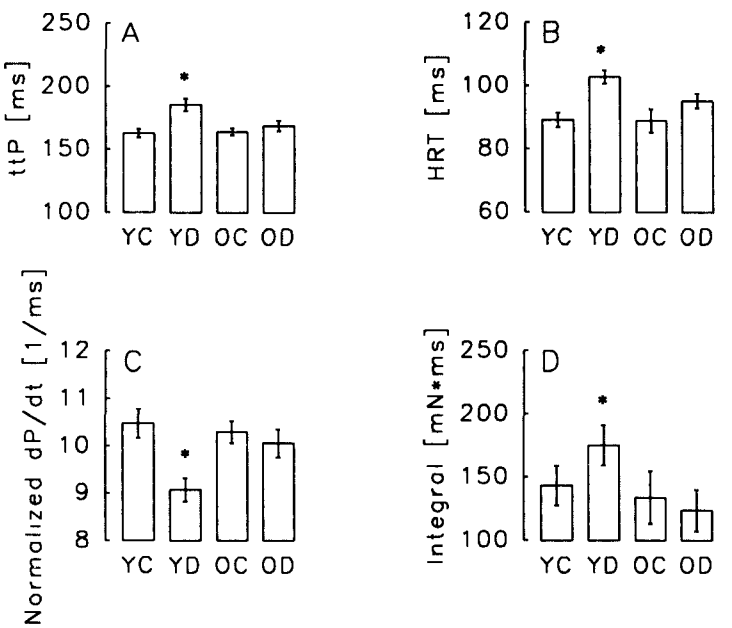

Figure 3. Changes of the contractile parameters in various types of experimental diabetes. *A* time to peak tension, *B* half relaxation time, *C* rate of rise of the contraction curve normalized to peak tension, *D* area under the contractile curve (integral). YC young control ($n = 13$), YD young diabetic ($n = 19$), OC old control ($n = 10$), OD old diabetic ($n = 13$). Columns and bars represent mean ± S.E.M. values. The asterisks denote significant difference from control ($p < 0.05$).

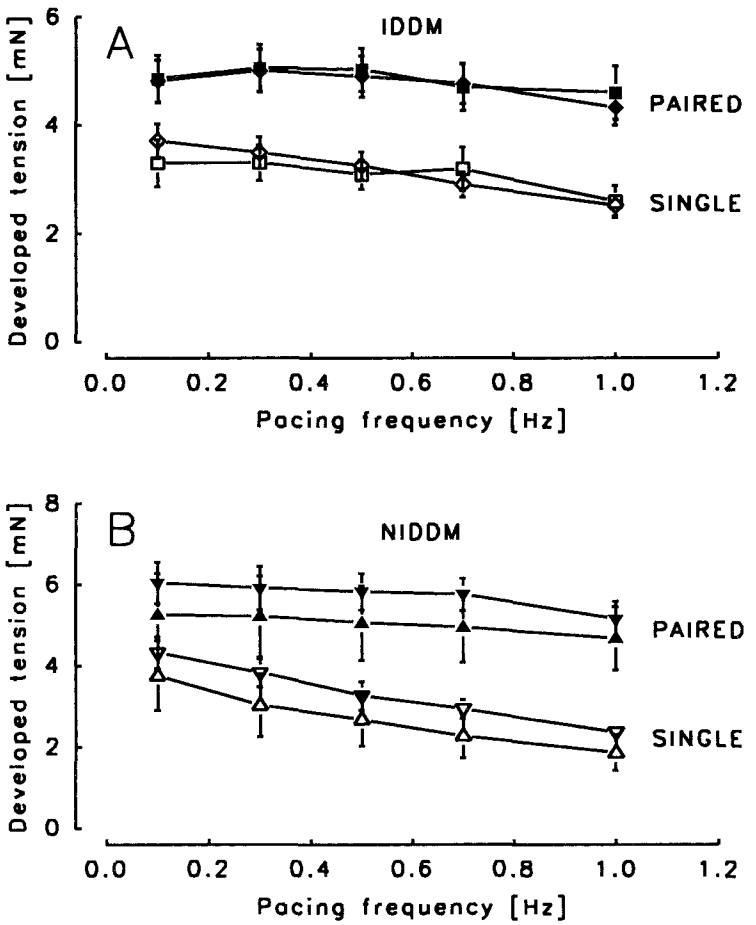

Figure 4. Force-frequency relationship of trabecular muscles obtained for IDDM (*A*) and NIDDM (*B*) preparations. Contractions were evoked by single (open symbols) and paired-pulse stimulation (filled symbols). Symbols and bars represent mean ± SEM values obtained from 9–16 preparations. Error bars smaller than the symbol size not shown. Squares: young control (YC); diamonds: young diabetic (YD); upward triangles: old control (OC); downward triangles: old diabetic (OD).

were prolonged in all IDDM animals (Fig. 3*A*,*B*). Changes in these parameters were always parallel: the ratio of *ttP* and *HRT* was not altered. In YD animals the velocity of tension development decreased (Fig. 3*C*) whereas the area under the curve increased significantly ($p < 0.05$) compared to the YC, OC or OD groups (Fig. 3*D*). In contrast to IDDM, NIDDM caused no significant changes in the contractile parameters. All the contractile parameter studied were identical in

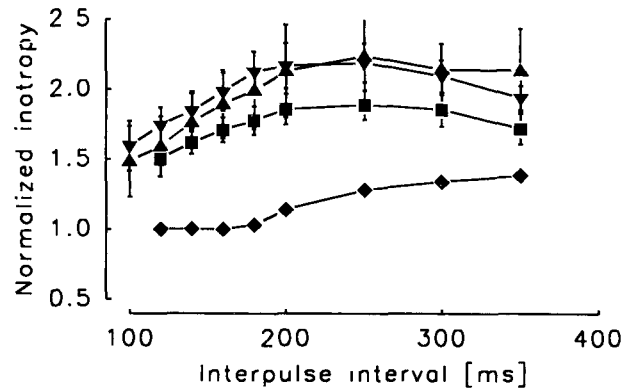

Figure 5. Interval-dependence of the paired-pulse induced motropic response in rat trabecular muscles measured at a constant pacing frequency of 0.5 Hz. Peak tension values obtained with paired-pulses were normalized to those recorded with single pulses prior to the application of the paired-pulse protocol. Same symbols as in Fig. 3. Each point represents mean ± S.E.M. obtained from 9–16 preparations.

the OC and OD groups (Fig. 3A – *D*). All the parameters selected to describe the contraction process were identical in the YC, OC and OD groups. Clearly the duration of the contractile process was identical in YC, OC and OD but it was increased in the YD group.

The contractile force of rat trabecular muscle decreased with the increasing of the stimulation frequency between 0.1 and 1 Hz in all groups examined (negative force-frequency relationship, Fig. 4). Continuous stimulation with paired-pulses resulted in positive motropic responses in each group independently of the pacing frequency or diabetic state. Similarly, the magnitude of the developed force was not significantly different in the control and diabetic animals. Nevertheless, the slope of the force-frequency relationship was greater in old animals (OC and OD) than in the young ones (YC and YD).

Fig. 5 shows the results obtained at a constant pacing frequency of 0.5 Hz using paired-pulse protocol with interpulse intervals ranging between 100 and 350 ms. Since the lengths and the diameters of the trabecular muscles varied within a wide range, the contractile force evoked by paired-pulses was divided by the contractile force obtained with single stimuli in order to decrease individual fluctuations. In the YD group both the interval-dependence and the degree of motropy were different from the respective parameters measured in the other groups. The application of paired-pulses with a 100 ms interpulse interval induced significant motropy in the YC, but not in the YD group. In young diabetic animals (YD), a minimum interval of 200 ms was required to increase contractility, however the degree of this motropy

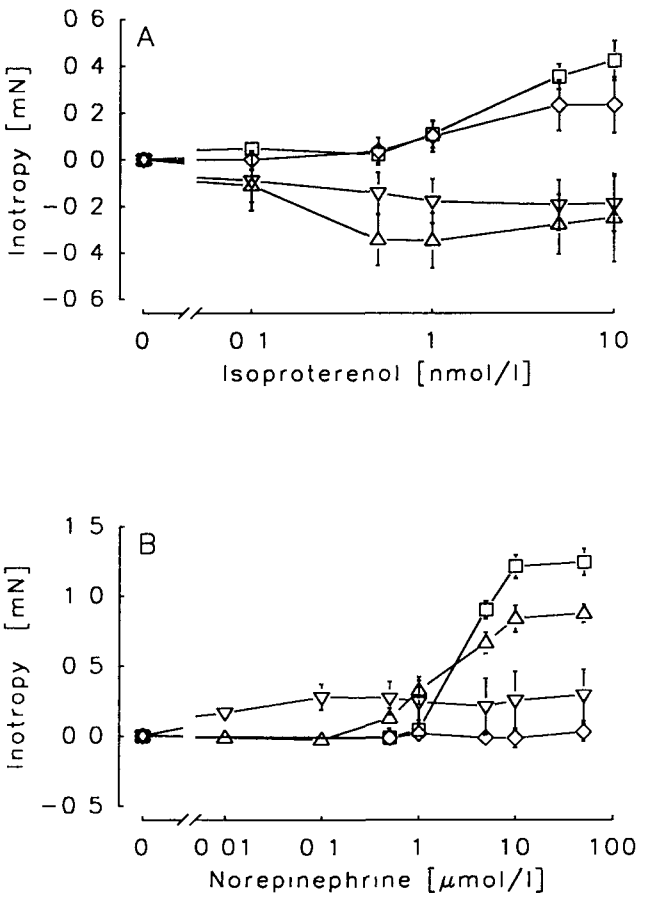

Figure 6. Cumulative dose-response curves obtained with isoproterenol (*A*) and norepinephrine (*B*) in diabetic rats. The magnitude of the inotropic response is given at the ordinate as the difference between the pre-drug and post-drug values. Symbols have similar meanings as in Fig. 4.

remained below the respective control level (YC). While the inotropic response in YC myocardium reached its maximum between 200 and 300 ms, inotropy in the YD animals continuously increased up to 400 ms. Animals of the old groups (OC and OD) displayed considerable similarity in their inotropic responses obtained with paired pulse protocol. There were no differences in the magnitude of the inotropic response and the interval dependence between the myocardium of old control (OC) and old diabetic (OD) animals.

Summarizing these results one might conclude that age and NIDDM have little

effect on contractility in rat trabecular muscle whereas the contraction process is altered markedly in IDDM. Using paired-pulses to induce motropy the interval-dependence was altered only in IDDM but not in NIDDM. In control animals the age had no effect on the contractile properties including the motropic response.

Concentration dependent effects of isoproterenol (ISO) and norepinephrine (NE) on the contractile force were also examined to study the motropic responses obtained by stimulation of alpha and beta adrenergic receptors in control and diabetic animals (Fig. 6A *B*). ISO induced positive motropic response in young (YC and YD) but negative motropy in old animals (OC and OD) independent of their diabetic state. In contrast to ISO, NE increased the contractility in control (YC and OC) but not in diabetic animals (OC and OD). This positive motropic response however was significantly smaller ($p < 0.05$) in the OC than in the YC group. Again old control animals were less sensitive to catecholamines than the young ones, since the degree of the catecholamine-induced positive motropy was smaller in the OC group.

Discussion

Our aim was to obtain information on the contractile properties of myocardium in healthy and diabetic rats. It was found that mechanical alterations of rat myocardium in IDDM and NIDDM are characteristically different.

When expressed in absolute values, the average developed forces (induced either by single or paired-pulse stimulation) were identical in the healthy and diabetic animals, as well as the negative force-frequency relationship characteristic to rat ventricular myocardium. Thus, our data are in accordance with the data reported by Fein et al. (1980) and also with those of Lopaschuk and Russel (1991) authors reporting no alterations in cardiac contractility in IDDM and NIDDM diabetes respectively. However, our data appear to conflict with the results of Schaffer and Wilson (1993) who reported decreased cardiac performance in NIDDM. This contradiction might be resolved with the introduction of altered myocardial compliance into the model of the working heart. NIDDM heart is known to have decreased compliance (Schaffer et al. 1985) resulting in decreased diastolic filling volume at a particular preload. Reduced end-diastolic volume with unaltered systolic pressure will necessarily lead to decreased cardiac work associated with a shift in the pressure-cardiac work relationship.

While the magnitude of the developed tension was similar in IDDM and NIDDM preparations, the time course of contraction was altered exclusively in IDDM preparations, similar to results reported from other laboratories (Rubinstein et al. 1984, Atkins et al. 1985, Heyliger et al. 1986, Rosen et al. 1986, Schaffer et al. 1989, Tanaka et al. 1992, Downing et al. 1993). This decreased velocity of contraction and relaxation may affect the interval-dependence of the paired-pulse induced

motropy. These results also indicate that the cellular mechanisms involved in the development of changes are basically different in IDDM and NIDDM. Schaffer et al. (1985) reported reduced myocardial contractility and relaxation in NIDDM, observations quite different from our results. Although this discrepancy might be due to the use of different biological preparations, it has to be emphasized that our results obtained in IDDM trabecular muscles, are in accordance with the previously published data obtained in isolated working heart (Fein et al. 1980, Rubinstein et al. 1984, Atkins et al. 1985, Heylinger et al. 1986, Rosen et al. 1986, Schaffer at al. 1989, Tanaka et al. 1992).

In the present report, catecholamines were shown to act unequally on IDDM and NIDDM myocardium. Although the motropic response was reduced in both types of diabetes, old control animals (OC) also displayed reduced sensitivity to ISO or NE, compared with young controls (YC). Decreased ISO sensitivity was reported earlier in IDDM (Atkins et al. 1985), however, no similar data are available for NIDDM preparations. The decreased catecholamine motropy may be due to either defective subsarcolemmal signalization or impaired force production of the myofilamental system. Diabetes-induced biochemical changes in the contractile proteins were reported in earlier studies. Increased fraction of V_3 myosin isoenzyme is known to be present in both forms of diabetes, as well as in non diabetic aged myocardium(Schauble et al. 1983, Rubinstein et al. 1984, Schaffer et al. 1985, Fozzard et al. 1986, Tahiliani and McNeil 1986). However, our data obtained with paired-pulse protocol suggest that diabetic myocardium is able to develop contractile force equal to the contraction of healthy muscle (see Fig. 3 *A*, *B*). Thus it appears that the decreased catecholamine sensitivity observed in diabetes may be due to changed receptor function or signalization rather than to alterations of the contractile proteins.

The interpretation of the concentration-dependent effects of ISO and NE is quite difficult since the results may be distorted by several time-dependent events such as receptor desensitization or exhaustion of the muscles. Similar difficulties arise when trying to explain the decreased catecholamine sensitivity observed in both forms of diabetes. The ISO resistance found in IDDM is consistent with the results of Sundaresan et al. (1984) who reported decreased beta receptor binding capacity in IDDM rats. No similar findings have been published for NIDDM. Our data suggest that reduced beta receptor density may be anticipated in non insulin-dependent diabetes mellitus as well. The ISO resistance found in the OD group was also present in the age matched controls (group OC). This similarity between OC and OD animals clearly shows that decreased ISO sensitivity found in NIDDM is more likely a result of aging than from the streptozotocin treatment itself. This hypothesis is supported by the results of Guarnieri at al. (1980) who found that the contractile response to catecholamines in the myocardium of senescent rats is diminished when compared to young adults. Our results also indicate that changes

in beta receptor function are different in the insulin-dependent and non insulin-dependent forms of diabetes mellitus however this difference may at least in part be attributed to aging

Age- or diabetes-dependent reduction in the density of alpha receptors have not been observed previously There were only moderate differences observed between the NE sensitivity of young and old controls (YC and OC) whereas both diabetic groups (YD and OD) displayed excessive NE resistance Therefore the decreased responsiveness to NE in both types of diabetes must be attributed to the effect of diabetes mellitus induced by STZ treatment

Summarizing our conclusions it can be suggested that both age and diabetes may contribute to the alpha and beta receptor disfunction observed in NIDDM However decreased beta receptor activity is a result of predominantly aging whereas alpha receptor disfunction is a result of chiefly the diabetic state

Acknowledgements. This work was supported by the Hungarian Academy of Sciences (OTKA-1166) We thank Dr Peter Nanasi for the constructive criticism of the manuscript Authors are grateful to Mrs K Horvath and Mrs K Laber for excellent technical assistance

References

Atkins J L Dowell R F Love S (1985) β-Adrenergic receptors adenylate cyclase activity and cardiac dysfunction in the diabetic rat J Cardiovasc Pharmacol **7**, 66 70

Downing S L Lee J C Tripp R R (1993) Enhanced sensitivity of diabetic hearts to α-adrenoceptor stimulation Amer J Physiol **245**, H808 H813

Fein F S Kornstein L B Strobeck J E Capasso J M Sonnenblick E H (1980) Altered myocardial mechanics in diabetic rats Circ Res **47**, 922 933

Fein F S Strobeck J E Malhotra A Scheuer J Sonnenblick E H (1981) Reversibility of diabetic cardiomyopathy with insulin in rats Circ Res **49**, 1251 1261

Fozzard H A Haber E Jennings R B Katz A M (1986) The Heart and Cardiovascular System Raven Press New York

Guarnieri T Filburn C R Ziltnik G Roth G S Lakatta E G (1980) Contractile and biochemical correlates of β-adrenergic stimulation of the aged heart Amer J Physiol **239**, H501 H508

Hevlinger C E Rodrigues B McNeil J H (1986) Effect of choline and methionine treatment on cardiac dysfunction of diabetic rats Diabetes **35**, 1152 1157

Lopaschuk G D Russel J C (1991) Myocardial function and energy substrate metabolism in the insulin resistant JCR LA corpulent rat J Appl Physiol **71**, 1302 1308

Penpargkul S Schaible E Yipintsoi T Scheuer J (1980) The effect of diabetes on performance and metabolism of rat hearts Circ Res **47**, 911 921

Rosen P Windeck P Zimmer H G Trenzel H Burig K F Reimann H (1986) Myocardial performance and metabolism in non-ketotic diabetic rat hearts myocardial function and metabolism *in vivo* and in the isolated perfused heart under the influence of insulin and octanoate Basic Res Cardiol **81**, 620 635

Rubinstein M Schaible T F Malhotra A Scheuer J (1984) Effects of graded insulin therapy on cardiac function in diabetic rats Amer J Physiol **246**, H153 H158
Saka M Danzinger R S Xiao R P Spurgeon H A Lakatta E G (1992) Contractile response of individual cardiac myocytes to norepinephrine declines with senescence Amer J Physiol **262**, H184–H189
Schaible T F Malhotra A Bauman W A Scheuer J (1983) Left ventricular function after chronic insulin treatment in diabetic and normal rats J Mol Cell Cardiol **15**, 145 158
Schaffer S W Wilson G L (1993) Insulin resistance and mechanical dysfunction in hearts of Wistar rats with streptozotocin-induced non-insulin-dependent diabetes mellitus Diabetologia **36**, 195 199
Schaffer S W Boen H I Wilson G L (1985) Development of cardiomyopathy in a model of noninsulin-dependent diabetes Amer J Physiol **248**, H179 H185
Schaffer S W Mozaffari M S Artman M Wilson G L (1989) Basis for myocardial mechanical defects associated with non-insulin dependent diabetes Amer J Physiol **256**, L25 I 30
Sundaresan P R Sharma V K Gingold S I Banerjee S P (1984) Decreased β adrenergic receptors in rat heart in streptozotocin induced diabetes role of thyroid hormones Endocrinology **114**, 1358 1363
Tahiliani A G McNeil J H (1986) Diabetes-induced abnormalities in the myocardium Life Sci **V38** 959 974
Tanaka Y Kashiwagi A Saeki Y Shigeta Y (1992) Abnormalities in cardiac α_1 adrenoceptor and its signal transduction in streptozotocin-induced diabetic rats Amer J Physiol **263**, E425 E 429
Verma S McNeil J H (1994) Metformin improves cardiac function in isolated streptozotocin diabetic rat hearts Amer J Physiol **266**, H714–H719
Warner I J Gradasso I Thompson C I Bellomi F I (1991) Vasodilative and anti-adrenergic effects of adenosin in diabetic rat hearts Can J Physiol **70**, 13 19
Xiang H McNeil J H (1991a) α_1 Adrenoceptor mediated phosphoinositide breakdown and inotropic responses in diabetic hearts Amer J Physiol **260**, H557 H562
Xiang H McNeil J H (1991b) Influence of arachidonic acid metabolites on cardiac α_1 adrenoceptor responses in diabetic rats Can J Physiol Pharmacol **70**, 680 686
Yu Z McNeil J H (1990) Altered inotropic responses in diabetic cardiomyopathy and hypertensive diabetic cardiomyopathy J Pharmacol Exp Ther **257**, 64 71
Zar J H (1984) Biostatistical Analysis Prentice Hall Inc Englewood Cliffs New Jersey

Final version accepted October 5 1996