Changes in Cardiac Contractility in IDDM and NIDDM Diabetic Rats

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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. Pared-pulse stimulation and catecholamine treatment were applied to compare the motropic 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 motropy was altered and the velocity of contraction and relaxation decreased in IDDM but not in NIDDM muscles. Sensitivity to isoproterenol and noradrenaline 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-
characterized 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, Heylinger 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 (Fem et al. 1980, Pennapargkul et al. 1980, Verma and McNeil 1994). In IDDM developed ventricular pressure was decreased with or without normal cardiac output (Pennapargkul et al. 1980, Rubinstein et al. 1984, Heylinger et al. 1986, Rosen et al. 1986, Schaffer et al. 1989, Tanaka et al. 1992, Verma and McNeil 1994) whereas NIDDM produced no change in the ventricular systolic pressure (Fem 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 (Fem et al. 1981) or increased (Xiang and McNeil 1991a) as well as unaltered (Fem et al. 1980) contractile force has been reported. Catecholamine induced motropy 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 stages of the disease. We desired to obtain information on the force-frequency relationship and the mototropic 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 motropy 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 motropy. Comparing these pharmacologically induced (catecholamine) and directly induced (paired-pulses) motropic 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 weekly. 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, MgCl₂ 1.5, CaCl₂ 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/cm²) 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 \( \text{tP} \), rising velocity of developed tension \( \text{dP/dt} \), half relaxation time \( \text{HRT} \)) and area under the contraction curve \( \text{Integral} \) 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 motropic 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 (Zai, 1981). Changes were considered significant when \( p \) was less than 0.05. Data are expressed as means ± SEM. A summary of the experimental groups and then 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>
<thead>
<tr>
<th>Group name</th>
<th>Abbreviation</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young diabetic</td>
<td>YD</td>
<td>4 months old rats with STZ-induced IDDM</td>
</tr>
<tr>
<td>Young control</td>
<td>YC</td>
<td>Age-matched control group of YD</td>
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<tr>
<td>Old diabetic</td>
<td>OD</td>
<td>12 months old rats with STZ-induced NIDDM</td>
</tr>
<tr>
<td>Old control</td>
<td>OC</td>
<td>Age-matched control group of OD</td>
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It should be noted that all animals were adults when used. Terms young and old refer to the different age of these animals (1 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 compared 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 ($t_{PP}$) and half relaxation time ($HRT$)

![Figure 1](image)

**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 ± SEM obtained from 27 animals.
Figure 2. The contractile parameters in various groups under the control and diabetic conditions.

Figure 3. The normalized $dP/dt$ and integral parameters in various groups under the control and diabetic conditions.

The data show significant differences from control animals. The asterisks indicate the statistical significance of the differences between the control and diabetic groups (\( \alpha < 0.05 \)).
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. 3A, B). Changes in these parameters were always parallel the ratio of tP and HRT was not altered. In YD animals the velocity of tension development decreased (Fig. 3C), whereas the area under the curve increased significantly ($p < 0.05$) compared to the YC, OC or OD groups (Fig. 3D). In contrast to IDDM, NIDDM caused no significant changes in the contractile parameters. All the contractile parameters 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 motropic response is given at the ordinate as the difference between the pre-drug and post-drug values. Symbols have similar meanings as in Fig 1.

remained below the respective control level (YC). While the motropic response in YC myocardium reached its maximum between 200 and 300 ms, motropy in the YD animals continuously increased up to 100 ms. Animals of the old groups (OC and OD) displayed considerable similarity in their motropic responses obtained with paired pulse protocol. There were no differences in the magnitude of the motropic 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 noradrenaline (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 same course of contraction was altered exclusively in IDDM preparations similar to results reported from other laboratories (Rubinstein et al. 1984 Atkins et al. 1985 Heyinger et al. 1986 Rosen et al. 1986 Schaffer et al. 1989 Tanaka et al. 1992 Downing et al. 1993). This decrease in 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 ob-
servations, 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 re-
results obtained in IDDM trabecular muscles, are in accordance with the previously
published data obtained in isolated working heart (Finn et al. 1980, Rubinstein et

In the present report, catecholamines were shown to act unequally on IDDM
and NIDDM myocardium. Although the mototropic 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 avail-
able 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 V1 myosin iso-
enzyme is known to be present in both forms of diabetes as well as in non diabetic
aged myocardium (Schaible et al. 1983, Rubinstein et al. 1984, Schaffer et al. 1985,
Pozzard et al. 1986, Tahiliani and McNeil 1986). However, our data obtained with
paced-pulse protocol suggest that diabetic myocardium is able to develop con-
tractile force equal to the contraction of healthy muscle (see Fig. 3A, 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 et 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.

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

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