Cardiac myofibrillar and sarcoplasmic reticulum function are not depressed in insulin-resistant JCR:LA-cp rats

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1Division of Stroke and Vascular Disease, St. Boniface General Hospital Research Centre, and Departments of 2Oral Biology and 3Physiology, University of Manitoba, Winnipeg, Manitoba R2H 2A6; and 4Department of Surgery, University of Alberta, Edmonton, Alberta, Canada T6G 2S2

Misra, Tarun, James S. C. Gilchrist, James C. Russell, and Grant N. Pierce. Cardiac myofibrillar and sarcoplasmic reticulum function are not depressed in insulin-resistant JCR:LA-cp rats. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1811–H1817, 1999.—Depressed myofibrillar Ca2+ -ATPase activity and sarcoplasmic reticulum (SR) Ca2+ uptake are important mechanisms that are responsible for the cardiac dysfunction exhibited by insulin-deficient (type I) diabetic animals. The JCR:LA-cp rat is a model for type II non-insulin-dependent diabetes mellitus (NIDDM). This rat is insulin resistant, obese, and has high levels of circulating glucose, cholesterol, insulin, and triglycerides. The purpose of this study was to determine whether changes in cardiac myofibrillar, SR, and cardiomyocyte function exist in this model of type II diabetes. Myofibrils and SR were isolated from hearts by differential centrifugation. Surprisingly, we found that myofibrillar Ca2+ -ATPase activities were unaltered in these animals. Ca2+ uptake in isolated SR fractions was increased in diabetic cp/cp rats, whereas Ca2+ -ATPase activity and ryanodine binding were unchanged. Cardiomyocytes isolated from hearts of control and experimental animals had similar active cell shortening and intracellular Ca2+ concentration under basal conditions and in response to caffeine. Our data argue against the presence of a cardiomyopathy in this diabetic model and suggest that insulin may be an important factor in the cardiomyopathy observed in type I diabetic models.

contractile proteins; calcium; diabetic cardiomyopathy; excitation-contraction coupling; myosin; cardiomyocytes; non-insulin-dependent diabetes mellitus; insulin

Diabetes mellitus induces cardiac dysfunction independent of vascular complications (5, 10, 28, 33, 36). The subcellular basis for the diabetic cardiomyopathy has received much research attention (see Refs. 5, 28, 33, and 38 for reviews). One subcellular organelle that has been implicated in the contractile dysfunction is the contractile protein. Myosin and myofibrillar ATPase activities have been closely correlated with tension development in the heart (44). Myofibrillar, actomyosin, and myosin ATPase activities are depressed in hearts from diabetic animals (2, 6–8, 20, 22, 23, 29, 30, 32, 34, 39, 43, 45). The sarcoplasmic reticulum (SR) is another subcellular organelle critical for cardiac performance and excitation-contraction coupling. Defects in SR Ca2+ uptake and release have been identified in hearts from diabetic animals (11, 27, 39, 43, 45, 46).

The vast majority of work done on the diabetic cardiomyopathy in animals has been carried out on insulin-deficient models of diabetes (chemically induced or Bio-Breeding Worcester rats). The animals typically have high circulating glucose and lipid levels, low circulating insulin concentrations, and depressed body weights (23, 29, 30, 34, 37). These characteristics resemble those of the type I, insulin-dependent diabetic state (24, 28). However, there is a relative lack of data on the more common form of diabetes, the insulin-resistant type II diabetes (non-insulin-dependent diabetes mellitus [NIDDM]). In type II diabetes, circulating levels of glucose are higher than normal despite elevated insulin concentrations, reflecting a peripheral insulin resistance. The syndrome is strongly associated with atherogenic dyslipidemia, obesity, and cardiovascular disease (9, 16, 19, 21). The JCR:LA-cp rat is an excellent alternative model of NIDDM that has advantages for the study of heart disease (9, 16, 19, 21). The unique strain exhibits a genetically determined development of insulin resistance and cardiovascular disease. Rats homozygous for the autosomal recessive cp gene (cp/cp) exhibit insulin resistance, glucose intolerance, hyperinsulinemia, and obesity, whereas heterozygous (+/cp) or homozygous normal (+/+) rats are unaffected (9, 19, 21, 40–42). The cp/cp rats are unusually sensitive to ischemic challenge to the heart (21). The cp/cp male rats have atherosclerotic lesions and exhibit spontaneous myocardial lesions (40–42). Of particular interest is the inability of hearts from these animals to function in the presence of high circulating Ca2+ concentrations ([Ca2+]i) (19). This raises the possibility that one or more of the subcellular organelles that respond to Ca2+ or that regulate intracellular [Ca2+]i may be defective within the myocardium of these rats. Changes in the Ca2+ sensitivity of the myofibrillar ATPase activity and/or the SR Ca2+ uptake in these hearts may explain the unusual response of these hearts to circulating Ca2+. Furthermore, a change in Ca2+-related function of these organelles would be entirely consistent with previous work in insulin-deficient models of diabetes (2, 6–8, 20, 22, 23, 29, 30, 34, 39, 45, 46). It is also unclear from previous studies whether the hearts from these animals exhibit defects...
in contractile performance under basal conditions. Conflicting data exist on this issue, with one study (19) demonstrating normal performance in the J CR:LA cp/cp rat hearts and another study (21) demonstrating a transient depression in contractile performance in these rat hearts. The purpose of the present study, therefore, was to examine the status of myofibrillar and SR function in hearts from J CR:LA-cp rats.

METHODS

Experimental animals. Male J CR:LA rats were bred and maintained until they reached 3 or 6 mo of age in the established breeding colony at the University of Alberta (9, 40–42). J CR:LA rats that are homozygous for the cp gene are obese, hyperphagic, insulin resistant, hyperinsulinemic, glucose intolerant, and hyperlipidemic (9, 40–42). J CR:LA animals that are heterozygous for the cp gene (+/cp) or homozygous normal (+/+) have a normal metabolic profile. Thus we have used heterozygous or homozygous normal animals as the lean control group (+/?). All care and treatment of the animals were in conformity with the Guidelines of the Canadian Council on Animal Care and subject to prior review by the appropriate institutional animal welfare committees. The cp/cp rats are infertile and difficult to obtain; therefore, experiments were carefully designed and kept to an absolute minimum. For this reason, the majority of experiments were carried out on 3-mo-old animals, and only in selected cases were 6-mo-old animals tested. By 3 mo of age, cp/cp rats had already exhibited insulin resistance and glucose intolerance for ~2 mo.

Biochemical measurements. Plasma cholesterol, glucose, and triglyceride were measured in the postprandial state as described in detail elsewhere (14). The final SR fraction was resuspended in 30% sucrose and 20 mM Tris (pH 7.0) and stored under a liquid N2 atmosphere before use. Oxalate-supported Ca2+ uptake, Ca2+-ATPase activity, and ryanodine binding in the SR vesicles were measured as described in detail previously (14).

Cardiomyocytes were isolated by standard collagenase digestion methodology as described elsewhere (18). The yield of rod-shaped, Ca2+-tolerant cardiomyocytes was ~70–80% in both groups. Myocyte contractile activity was monitored in an unloaded setting with a video edge-detection system (Crescent Electronics, Sandy, UT) coupled to a Pulnix monochrome charge-coupled device camera. Cell shortening was monitored during electrical stimulation with platinum electrodes at a rate of 0.5 Hz with 200-ms duration. A microscope stage micrometer was used to calibrate the cell length. Cells were perfused in a Leiden chamber on the microscope stage at a rate of 1 ml/min with a perfusate heated to 37°C and bubbled extensively with 100% O2. The perfusate contained 140 mM NaCl, 6 mM KCl, 1 mM MgCl2, 1.25 mM CaCl2, 6 mM HEPES (pH 7.4), 10 mM dextrose, and 0.02% bovine serum albumin. Cells were equilibrated in this solution for a period of time before the start of any experimental interventions.

Intracellular [Ca2+] was measured spectrofluorometrically using the Ca2+ indicator dye fura 2 (Molecular Probes, Eugene, OR) as previously described (18). In the system described above, a SPEX Fluorolog spectrophotometer was attached to a Nikon Diaphot epifluorescent microscope. Photomultiplier tubes were coupled to a Pentium computer for quantitation of the fluorescent signal. Calibration of the signal was carried out with a microscope stage micrometer.

Statistical analyses. A two-tailed Student’s t-test was used to measure statistical significance (P < 0.05).

RESULTS

Animal characteristics and plasma metabolite levels. Male cp/cp rats exhibited higher body, heart, and liver weights than their corresponding lean +/+ control counterparts at 3 mo of age (Table 1). Similar trends exist for older animals (data not shown). The heart-to-body weight ratio was lower in the cp/cp rats compared with those in the age-matched, lean control animals. A similar trend was observed in another model of NIDDM (32). Conversely, despite the large increase in body mass, the liver-to-body weight ratio was maintained in the cp/cp rats.

Plasma metabolite characteristics for these animals are similar to those reported elsewhere (9, 19, 21, 22).

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Wt, g</th>
<th>Heart Wt, g</th>
<th>Heart Wt/Body Wt Ratio, mg/g</th>
<th>Liver Wt, g</th>
<th>Liver Wt/Body Wt Ratio, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>330 ± 6</td>
<td>0.77 ± 0.01</td>
<td>2.35 ± 0.06</td>
<td>10.0 ± 0.2</td>
<td>30.3 ± 0.8</td>
</tr>
<tr>
<td>cp/cp</td>
<td>496 ± 6*</td>
<td>0.98 ± 0.02*</td>
<td>1.98 ± 0.03*</td>
<td>17.3 ± 1.0*</td>
<td>34.9 ± 1.7</td>
</tr>
</tbody>
</table>

Values represent means ± SE for 3-mo-old animals (n = 4–8 animals) in each group. Body wt, weight; +/+, lean rats; cp/cp, corpulent rats. *P < 0.05 vs. age-matched lean group.
Plasma cholesterol, glucose, and triglyceride levels were elevated in the cp/cp rats compared with those in the corresponding age-matched control animals (Table 2).

Resting serum insulin levels were elevated in corpulent rats compared with those in control animals (Fig. 1). The cp/cp rats also exhibited markedly higher insulin levels in immediate response to the ingestion of a defined meal than the corresponding control animals. These levels remained elevated for the entire 3-h period of study after ingestion of the meal.

Myofibrillar characteristics and activities.

Myofibrillar yield for the 1/1 control rats was 73 ± 6 and 59 ± 6 mg/g wet tissue weight for the 3- and 6-mo-old rats, respectively, and 63 ± 2 and 63 ± 3 mg/g wet tissue weight for the 3- and 6-mo-old cp/cp rats, respectively (n = 8). There were no differences between the genotypes (P > 0.05).

Purity of the myofibrillar protein fractions was estimated by measuring typical marker enzyme activities from sarcolemmal membranes, mitochondria, and SR. Na\(^+\)-K\(^+\)-ATPase, succinic dehydrogenase, and mannose-6-phosphatase activities were undetectable in the myofibrillar fractions from all groups. Furthermore, SDS-polyacrylamide gel analysis revealed no detectable differences in the protein profile between the myofibrillar fractions.

Myofibrillar Ca\(^{2+}\)-ATPase activities were measured as a function of reaction time in cardiac myofibrils isolated from 3-mo-old cp/cp and +/+ rats (Fig. 2A). There were no significant differences in activities between the two groups (P > 0.05). It is possible that the duration of diabetes was insufficient to induce changes in the function of the cardiac subcellular organelles. For example, hearts of JCR:LA-cp rats respond very differently to an ischemic insult, depending on the age of the animals (3 or 6 mo old) (21). Insulin and glucose abnormalities are apparent at ~1 mo of age in the JCR:LA-cp rats (data not shown). To investigate the possibility that the duration of diabetes is an important factor, cardiac myofibrillar Ca\(^{2+}\)-ATPase activities were studied in rats that were 6 mo old. There were no significant differences in activity over 1–20 min of reaction time (Fig. 2B).

Cardiac myofibrillar ATPase activity was measured as a function of varying [Ca\(^{2+}\)] (Fig. 3). Increasing [Ca\(^{2+}\)] stimulated ATPase activity. Maximal ATPase activity was observed at ~8 × 10\(^{-6}\) M Ca\(^{2+}\). Half-maximal activation was observed at ~2.5 × 10\(^{-6}\) M Ca\(^{2+}\). There were no significant differences in the ATPase activity as a function of [Ca\(^{2+}\)] between groups in 3-mo-old animals (Fig. 3A). Furthermore, we could

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol, µmol/ml</th>
<th>Glucose, mg/dl</th>
<th>Triglycerides, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>0.48 ± 0.05</td>
<td>155 ± 6</td>
<td>59 ± 10</td>
</tr>
<tr>
<td>cp/cp</td>
<td>1.14 ± 0.11*</td>
<td>278 ± 32*</td>
<td>186 ± 31*</td>
</tr>
</tbody>
</table>

Values represent means ± SE for 4–5 animals in each group. *P < 0.05 vs. age-matched lean group.

Fig. 1. Serum insulin levels in JCR:LA-cp lean control (+/; ○) and corpulent (cp/cp; ●) rats before and after ingestion of a test meal of 5 g of rat chow at time 0. Values represent means ± SE for 7 animals in each group.

Fig. 2. Myofibrillar Ca\(^{2+}\)-ATPase activity as a function of reaction time in hearts from 3- (A) and 6-mo-old (B) JCR:LA lean control (○) and corpulent (●) rats. Values represent means ± SE for 4–6 samples. There were no significant differences between genotypes (P > 0.05).
not detect any significant differences in Ca\textsuperscript{2+}-ATPase activity as a function of [Ca\textsuperscript{2+}] in 6-mo-old rats (Fig. 3B).

The ATPase activity did exhibit a modest but statistically insignificant shift to the right in Ca\textsuperscript{2+} sensitivity. The Hill coefficients from plots of these data for the lean and corpulent groups are 1.22 and 1.44, respectively. The EC\textsubscript{50} for the two sets of data are 2.2 and 2.5 µM Ca\textsuperscript{2+} for the lean and corpulent groups, respectively. Thus myofibrillar ATPase activities were not altered in JCR:LA-cp rats that were 3 or 6 mo old.

Cardiac SR function and characteristics. The SR is another subcellular organelle that is critical for cardiac contractile performance. SR Ca\textsuperscript{2+} uptake contributes to force generation and relaxation (15, 28) and is defective in hearts from insulin-dependent diabetic animal models (11, 27, 39, 43, 45, 46). SR vesicles were isolated from hearts from 3-mo-old JCR:LA-cp rats and examined for oxalate-supported Ca\textsuperscript{2+} uptake as a function of assay reaction time (Fig. 4).

Surprisingly, there was a modest but significant increase in SR Ca\textsuperscript{2+} uptake in diabetic cp/cp rats compared with that in the +/+ control group.

SR Ca\textsuperscript{2+} uptake occurs through the activity of the SR Ca\textsuperscript{2+}-ATPase. SR Ca\textsuperscript{2+}-ATPase activity was examined in hearts from lean and corpulent rats in the absence or presence of varying [Ca\textsuperscript{2+}]. No significant change in SR Ca\textsuperscript{2+}-ATPase activity was observed under any of the experimental conditions (Fig. 5).

SR Ca\textsuperscript{2+} release is regulated through ryanodine-sensitive Ca\textsuperscript{2+}-release channels (14, 46). These channels can be quantitated with radiotopic assays for [\textsuperscript{3}H]ryanodine binding. Ryanodine binding sites are depressed in myocardial homogenates obtained from insulin-dependent diabetic rats (46). However, in SR isolated from +/+ and cp/cp rat hearts, specific ryanodine binding was unchanged (Fig. 6).

It is possible that the results obtained on subcellular organelles may not accurately reflect activities at a cellular level; therefore, single cardiomyocytes were isolated from JCR:LA-cp rats and monitored for active cell shortening. There were no significant differences in active cell shortening between the two groups in response to electrical stimulation (data not shown). Even if the electrical stimulation was varied from 0.5 to 1.0 Hz, no differences in active cell shortening were observed between the two groups (data not shown). To confirm the results obtained with the isolated SR vesicles, we also examined the response of the cells when perfused with 10 mM caffeine. Caffeine is capable of increasing contractile activity by releasing Ca\textsuperscript{2+} from SR stores. As shown in Fig. 7, there were no significant differences in active cell shortening in response to 10 mM caffeine between cells from the two groups. Significant differences in resting cell length were detected.
only at two early time points after exposure to caffeine (Fig. 8). However, all other time points examined were similar in the two groups.

Intracellular \([\text{Ca}^{2+}]\) was studied in response to electrical stimulation in cardiomyocytes isolated from the two groups of rats. Diastolic cell \([\text{Ca}^{2+}]\) was 202 ± 17 and 211 ± 36 nM in lean and corpulent rat cells, respectively (\(P > 0.05\)). Systolic cell \([\text{Ca}^{2+}]\) was 335 ± 28 and 428 ± 46 nM in lean and corpulent rat cardiomyocytes, respectively (\(P > 0.05, n = 9–22\)). The effect of caffeine on cell \([\text{Ca}^{2+}]\) was also studied. As shown in Fig. 9, caffeine induced a decrease in the intracellular \([\text{Ca}^{2+}]\) transient. This was expected from previous work (15). This effect is due to an ability of caffeine to increase myofilament sensitivity to \([\text{Ca}^{2+}]\) (15). Consistent with the cell shortening data, there were essentially no significant differences between the two groups in the response of cellular \([\text{Ca}^{2+}]\) to caffeine. Only one of the data points was significantly different between the two groups.

DISCUSSION

The present study demonstrates a lack of depression in myocardial myofibrillar and SR function in the cp/cp rats compared with the +/+ control JCR:LA-cp rats despite the presence of diabetes. This result is surprising in view of the number of studies that have reported a depression in contractile protein ATPase activity (2, 6–8, 20, 22, 23, 29, 30, 32, 34, 39, 43, 45) and SR \([\text{Ca}^{2+}]\) uptake (11, 27, 39, 45, 46) in insulin-deficient, type I diabetic rat hearts. We have confidence in the validity of our results from several perspectives. Our results were not confounded by differential contamination of the myofibrils or SR by other subcellular fractions. The activities for myofibrillar ATPase and SR \([\text{Ca}^{2+}]\) uptake as...
a function of varying [Ca$^{2+}$] and reaction time; ryanodine binding; cell shortening and [Ca$^{2+}$] as a function of caffeine) and the types of activities measured (myofibril, SR, isolated cells) produced a consistent pattern of results. Indeed, in one case there was actually an increase in SR Ca$^{2+}$ uptake in hearts from cp/cp rats (Fig. 5). This increase was not large but may have been significant for some of the isolated changes in cell shortening and [Ca$^{2+}$] in response to caffeine as shown in Figs. 8 and 9. Furthermore, the age of the JCR:LA-cp rats did not influence the results. This is particularly important because the cardiac response to ischemic injury has been shown to change with age (21). All of these experiments consistently demonstrate that myofibrillar and SR functions were not depressed in the hearts from diabetic cp/cp rats compared with these functions in the control +/− rats.

Our results appear to be difficult to reconcile with the fact that so many previous studies have reported a significant depression in myocardial contractile protein ATPase activity (2, 6–8, 20, 22, 23, 29, 30, 32, 34, 39, 43, 45) and SR function (11, 27, 39, 45, 46) during diabetes. The most obvious difference between our work and the majority of these previous studies is that the animals in the present study exhibited an insulin-resistant, hyperinsulinemic, type II diabetic state, not an insulin-deficient, type I diabetic state. Our data would suggest that myocardial contractile protein defects and SR Ca$^{2+}$ transport lesions do not necessarily accompany diabetes where it is defined by glucose intolerance and hyperinsulinemia. Instead, the type of diabetes mellitus (IDDM vs. NIDDM) may be critical for the generation of the myocardial dysfunction. Several studies have reported depressions in contractile protein ATPase activity, SR Ca$^{2+}$ transport (32, 43), and cell [Ca$^{2+}$] (1) in NIDDM models. However, the animal models used in these studies have important limitations that may restrict the conclusions. Neither of the other two models of NIDDM (1, 32, 43) had high circulating basal insulin levels. Furthermore, the chemically induced model of NIDDM exhibits normal body weight, which contrasts with the majority of patients with type II diabetes, who are obese (24). These two factors may represent important metabolic differences. Clearly, the strength of the present study lies in the animal model employed. The abdominal obesity, insulin resistance, and high risk of cardiovascular disease observed in the JCR:LA-cp rat closely mimics the clinical state seen in humans. The rats develop atherosclerotic lesions in the heart, suffer from spontaneous small infarcts, and have a metabolic profile (glucose, lipid, and hormonal) remarkably similar to that of humans with NIDDM (9, 19, 21, 40–42). Our results do not explain the contractile dysfunction exhibited by JCR:LA-cp rats in response to high circulating [Ca$^{2+}$] (11). It is possible that thin filament sensitivity to Ca$^{2+}$ may be altered. We did not measure myosin isoform changes. Furthermore, other Ca$^{2+}$ regulatory systems such as the sarcolemma membrane may play a role in this defect. It is also important to recognize that the cell-shortening data are limited because the cells were measured in an unloaded state. However, previous studies (19) in Langendorff perfused hearts also demonstrated no significant changes in contractile performance in the JCR:LA cp/cp rats.

Our data suggest that lipid abnormalities and hyperglycemia per se are not mechanistic factors likely to explain the presence of a cardiomyopathy in the insulin-deficient diabetic animals. The JCR:LA-cp rat exhibits both of these metabolic abnormalities and still does not display the myocardial dysfunction typically observed in insulin-deficient diabetic animals. Instead, the cp/cp rat exhibits higher circulating lipid levels than insulin-deficient diabetic animals. Instead, chronic insulin deficiency may play an important role in depressing cardiac contractile function in insulin-deficient diabetes. It is well known that insulin treatment can reverse the defects in myofibrillar and SR function in insulin-deficient diabetic rats (11, 12, 20). Insulin can directly increase SR Ca$^{2+}$ uptake (31) and can alter ATPase sensitivity to Ca$^{2+}$ (17), but it has no direct effect on myofibrillar function (31). It is important to note that compounds similar to insulin such as insulin-like growth factor-1 have the capacity to induce significant improvements in the mechanical characteristics of ventricular myocytes when overexpressed in these cells (35). These changes were associated with alterations in contractile proteins (35). It is possible, therefore, that a chronic deficiency of insulin may induce a cardio-depressive state, whereas chronic hyperinsulinemia may protect against this metabolic lesion. However, this remains to be proven, and our results are only suggestive on this matter at the present time.

Our data reinforce the contention (33, 42) that cardiovascular disease in the JCR:LA-cp diabetic model is a vascular problem, not a cardiac one. The opposite holds for insulin-deficient diabetic rats. It is tempting to speculate that type I and type II diabetes in humans may also be very different with respect to their expression of primary cardiac lesions.

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