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

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Division of Stroke and Vascular Disease, St. Boniface General Hospital Research Centre, and Departments of Oral Biology and Physiology, University of Manitoba, Winnipeg, Manitoba R2H 2A6; and Department 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 Ca\(^{2+}\)-ATPase activity and sarcoplasmic reticulum (SR) Ca\(^{2+}\) 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 Ca\(^{2+}\)-ATPase activities were unaltered in these animals. Ca\(^{2+}\) uptake in isolated SR fractions was increased in diabetic cp/cp rats, whereas Ca\(^{2+}\)-ATPase activity and ryanodine binding were unchanged. Cardiomyocytes isolated from hearts of control and experimental animals had similar active cell shortening and intracellular Ca\(^{2+}\) 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.

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 Ca\(^{2+}\) 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 (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 abdominal or android obesity (24). Only a few studies (21, 32, 43) have examined cardiac integrity and function in a model of type II diabetes, and there are no studies examining cardiac subcellular function in a genetic model of type II diabetes.

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, 40–42). This 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, elevated circulating lipid levels, 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 atherosclerosis 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 Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]) (19). This raises the possibility that one or more of the subcellular organelles that respond to Ca\(^{2+}\) or that regulate intracellular Ca\(^{2+}\) may be defective within the myocardium of these rats. Changes in the Ca\(^{2+}\) sensitivity of the myofibrillar ATPase activity and/or the SR Ca\(^{2+}\) uptake in these hearts may explain the unusual response of these hearts to circulating Ca\(^{2+}\). Furthermore, a change in Ca\(^{2+}\)-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 previously described (29, 30, 32). Insulin levels were also measured in response to a defined meal. Rats were deprived of food overnight and bled from the tip of the tail for an initial quantitation of the fluorescent signal. Calibration of the multiplier tubes were coupled to a Pentium computer for quantitation of the fluorescent signal. Calibration of the signal was carried out with a micros scope stage micrometer. 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 spectrofluorometer 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 to their counterparts at 3 mo of age (Table 1). Similar trends were 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.

Table 1. Selected morphometric characteristics of J CR:LA cp/ cp and +/+ rats

<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. 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.

<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.

Myofibrillar characteristics and activities. Myofibrillar yield for the +/- control rats was 73 ± 8 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...
not detect any significant differences in Ca\(^{2+}\)-ATPase activity as a function of [Ca\(^{2+}\)] in 6-mo-old rats (Fig. 3).

The ATPase activity did exhibit a modest but statistically insignificant shift to the right in Ca\(^{2+}\) sensitivity. The Hill coefficients from plots of these data for the lean and corpulent groups are 1.22 and 1.44, respectively. The EC\(_{50}\) for the two sets of data are 2.2 and 2.5 µM Ca\(^{2+}\) 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\(^{2+}\) 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\(^{2+}\) uptake as a function of assay reaction time (Fig. 4). Surprisingly, there was a modest but significant increase in SR Ca\(^{2+}\) uptake in the diabetic \(cp/cp\) rats compared with that in the \(+/+\) control group.

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

SR Ca\(^{2+}\) release is regulated through ryanodine-sensitive Ca\(^{2+}\)-release channels (14, 46). These channels can be quantitated with radioligand assays for \(^{3}H\)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\(^{2+}\) 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 $202 \pm 17$ and $211 \pm 36$ nM in lean and corpulent rat cells, respectively ($P > 0.05$). Systolic cell $[\text{Ca}^{2+}]$ was $335 \pm 28$ and $428 \pm 46$ 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, 32, 34, 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 in control animals are similar to those reported previously (25, 29, 30, 32). The number of different measurements undertaken (ATPase activity and $\text{Ca}^{2+}$ uptake as...
important because the cardiac response to ischemic
in Figs. 8 and 9. Furthermore, the age of the JCR:LA-
shortening and [Ca2+] in response to caffeine as shown
in Figs. 8 and 9. Furthermore, the age of the JCR:LA-
results. Indeed, in one case there was actually an
increase in SR Ca2+ uptake in hearts from cp/cp rats
(Fig. 5). This increase was not large but may have been
responsible for some of the isolated changes in cell
shortening and [Ca2+] as a function of caffeine (1) in NIDDM models. However, the animal
studies (19) in Langendorff perfused hearts also demon-
strated no significant changes in contractile performance
in the JCR:LA cp/cp rats.
Our data suggest that lipid abnormalities and hyper-
glycemia per se are not mechanistic factors likely to
explain the presence of a cardiomyopathy in the insulin-
deficient diabetic animals. The J CR:LA-cp rat exhibits
each 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 even higher circulating lipid levels than
insulin-deficient diabetic animals. Instead, chronic in-
sulin deficiency may play an important role in de-
pressing cardiac contractile function in insulin-defi-
cient 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 Ca2+ uptake (31) and can alter
ATPase sensitivity to Ca2+ (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 signifi-
cant 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 cardio-
vascular disease in the J CR: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 expres-
sion of primary cardiac lesions.

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