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.

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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 measurement. Rats were deprived for 4–8 mo. Further presented with a test meal consisting of a 5-g pellet of rat chow that was consistently eaten immediately. Further 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.

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.

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/ Body Wt Ratio, mg/g</th>
<th>Liver Wt, g</th>
<th>Liver/ Body Wt Ratio, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>cp/cp</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>+/+</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.

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

### Table 2. Cholesterol, glucose, and triglyceride levels in plasma from JCR:LA cp/cp and +/+ rats

<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.
not detect any significant differences in Ca$^{2+}$-ATPase activity as a function of [Ca$^{2+}$] in 6-mo-old rats (Fig. 3B). 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 J CR: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 ± 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 \(\text{cp/cp}\) rats compared with the \(+/?\) control JCR:LA-\(\text{cp}\) 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; ryana
dine binding; cell shortening and [Ca\(^{2+}\)] as a function of
caffeine) and the types of activities measured (myofi-
bril, 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
responsible 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 J CR:LA-cp
rats did not influence the results. This is particularly
important because the cardiac response to ischemic
insult in J CR:LA-cp rats 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 de-
fects and SR Ca\(^{2+}\) transport lesions do not necessarily
accompany diabetes where it is defined by glucose
tolerance and hyperinsulinemia. Instead, the type of
diabetes mellitus (IDDM vs. NIDDM) may be critical
for the generation of the myocardial dysfunction. Sev-
eral 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 limita-
tions that may restrict the conclusions. Neither of the
other two models of NIDDM (1, 32, 43) had high
circulating basal insulin levels. Furthermore, the chemi-
cally 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 J CR:LA-cp rat closely mimics the
clinical state seen in humans. The rats develop athero-
sclerotic 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
J CR: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 isozyme
changes. Furthermore, other Ca\(^{2+}\) regulatory systems
such as the sarcolemmal 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 demon-
strated no significant changes in contractile perfor-
ance in the J CR: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
both of these metabolic abnormalities and still does not
display the myocardial dysfunction typically observed
in insulin-deficient diabetic animals. Indeed, 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 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 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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