Characterization of Contractile Function in Diabetic Hypertensive Cardiomyopathy in Adult Rat Ventricular Myocytes

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Diabetes and hypertension are major risk factors leading to increased cardiovascular mortality and morbidity.1,2 Accumulating evidence has demonstrated the existence of independent and specific cardiomyopathies associated with both diabetes and hypertension. Diabetic cardiomyopathy, a myopathic state independent of macrovascular complications, is the leading cause of death in diabetes.3,4 Diastolic dysfunction is the most prominent defect of diabetic cardiomyopathy, and is manifested by decreased compliance, prolonged myocardial relaxation and altered intracellular Ca²⁺ homeostasis.5-7 On the other hand, hypertensive, or hypertrophic cardiomyopathy, is characterized by myocardial hypertrophy, disorganization of cardiac myocytes and myofilaments.8 Hypertrophic cardiomyopathy also exhibits abnormalities in diastolic function including prolonged relaxation, reduced...
rate of rapid filling and increased left ventricular stiffness. Clinical and experimental evidence has suggested that although hypertrophic cardiomyopathy is a direct result of elevated blood pressure, it may also be associated with insulin resistance, hyperinsulinemia, and altered adrenergic responsiveness.

Hypertension aggravates the cardiovascular complications associated with diabetes and vice versa. Concurrence of the two disorders results in more devastating structural and functional cardiac impairments than are caused by either disease alone, and often leads to premature congestive heart failure, sudden cardiac death, and acute myocardial infarction. It has been postulated that the structural myocardial damage in concurrent diabetes and hypertension may be attributed primarily to hypertension whereas the myocellular dysfunction primarily to diabetes. The combination of diabetes and hypertension impairs the myocardial contractile function synergistically, and leads to sufficient organ damage to induce a severe, potentially fatal diabetic hypertensive cardiomyopathy.

Studies on diabetic hypertensive cardiomyopathy have so far been limited to the whole heart and multicellular tissue levels. In an attempt to characterize the association between the two myopathic states at the cellular level, we examined the cardiac contractile function in ventricular myocytes from diabetic, spontaneously hypertensive rats. The diabetic hypertensive rat is characterized by myocardial hypertrophy and fibrosis, microvascular pathology, pulmonary congestion, a high mortality, and has proven to be a new experimental model provoking myocardial and vascular deterioration similar to that seen in humans.

**Materials and Methods**

**Animals**

All animal experimentation was conducted in accord with humane animal care standards outlined in the *NIH Guide for the Care and Use of Experimental Animals*. Briefly, 7-month-old male spontaneously hypertensive rats (SHR) and their normotensive controls Wistar-Kyoto (WKY) rats (Harlan Sprague-Dawley, Indianapolis, IN, USA) were made diabetic with a single intravenous tail vein injection of streptozotocin (STZ, 55 mg/kg) as described and maintained for 8 weeks of untreated diabetes. A group of sham-treated age-matched euglycemic WKY and SHR rats were selected in parallel with the STZ-treated rats. The diabetic state was assessed by measurement of the glucose concentration in serum samples collected at the time of heart removal. Serum was separated by low speed centrifugation and stored at −20°C for analysis of glucose with a glucose monitor (Accu-ChekII, model 792, Boehringer Mannheim Diagnostics, Indianapolis, IN, USA). Systolic blood pressure was measured weekly using a semi-automated, amplified tail-cuff device (IITC Inc., Woodland Hills, CA, USA).

**Cell isolation procedures**

Single ventricular myocytes were enzymatically isolated from the hearts using the method described previously. Briefly, hearts were rapidly removed and perfused (at 37°C) with Krebs-Henseleit (KHB) buffer containing (in mM): 118 NaCl, 4.7 KCl, 1.25 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25 NaHCO₃, 10 N-[2-hydroethyl]-piperazine-N’-[2-ethanesulfonic acid] (HEPES) and 11.1 glucose, equilibrated with 5% CO₂-95% O₂. Hearts were subsequently perfused with a nominally Ca²⁺-free KHB buffer for 2–3 min until spontaneous contractions ceased followed by a 20 min perfusion with Ca²⁺-free KHB containing 223 U/ml collagenase (Worthington Biochemical Corp., Freehold, NJ, USA) and 0.1 mg/ml hyaluronidase (Sigma Chemical, St Louis, MO, USA). After perfusion, ventricles were removed and minced, under sterile conditions, and incubated with the calcium-free KHB with collagenase solution for 3–5 min. The cells were further digested with 0.02 mg/ml trypsin (Sigma) before being filtered through a nylon mesh (300 μm) and subsequently separated from the collagenase-trypsin solution by centrifugation (60 × g for 30 s). Myocytes were resuspended in a sterile-filtered, Ca²⁺-free Tyrode’s buffer containing (in mM): 131 NaCl, 4 KCl, 1 MgCl₂, 10 HEPES, and 10 glucose, supplemented with 2% bovine serum albumin, with a pH of 7.4 at 37°C. Cells were initially washed with Ca²⁺-free Tyrode’s buffer to remove residual enzyme and extracellular Ca²⁺ was slowly added back to 1.25 mM. Only rod-shaped myocytes with clear edges were selected for recording of mechanical properties as previously described.

**Cell shortening/relengthening**

Mechanical properties of ventricular myocytes were assessed using a video-based edge-detection system.
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In brief, amplitude (PS) normalized to CL in left ventricular myocytes was not affected by hypertension alone but significantly enhanced by diabetes. Interestingly, interaction of diabetes and hypertension significantly reduced PS. Myocytes under sustained hypertension (SHR) demonstrated significantly prolonged time-to-90% relengthening (TR 90), associated with normal time-to-peak shortening (TPS), compared to its WKY counterparts. Sustained diabetes (WKY-STZ and SHR-STZ) significantly prolonged both TPS and TR 90 (Fig. 1). Lastly, the maximal velocities of shortening (+dL/dt) and relengthening (−dL/dt) were significantly reduced in both hypertensive groups (SHR and SHR-STZ). Diabetes alone exerted no effect on +dL/dt (Fig. 2). These data suggest that concomitant diabetes and hypertension is capable of eliciting an additive effect on certain (such as PS) but not all cardiac contractile parameters compared to either disease alone.

Effect of extracellular Ca$^{2+}$ on myocyte shortening

The effect of extracellular Ca$^{2+}$ on myocyte shortening was examined and is shown in Figure 3. Increases in extracellular Ca$^{2+}$ concentration from 0.5 mM to 3 mM resulted in a positive staircase in myocyte shortening response in WKY, SHR, SHR-STZ but not the WKY-STZ group, indicating that the myocytes from the diabetic group were less sensitive to increases in extracellular Ca$^{2+}$. These data may suggest a possible alteration in myocyte responsiveness to Ca$^{2+}$ in diabetes, which has been reported previously in both chemically-induced and genetically-predisposed diabetic models. In diabetes, as the SHR-STZ group exhibited a normal Ca$^{2+}$ responsiveness.

Effect of stimulation frequency on myocyte shortening

Rat hearts normally contract at very high frequencies (300 beat/min), whereas our baseline studies were conducted at 0.5 Hz. To look for possible derangement of cardiac E-C coupling at higher frequencies, we increased the stimulating frequency up to 5 Hz (300 beat/min) and recorded the steady-state peak shortening. Cells were initially stimulated to contract at 0.5 Hz for 5 min to ensure steady-state before commencing the frequency study. All the recordings were normalized to PS at 0.1 Hz of the same myocyte. Figure 4 shows a negative

Statistical analyses

For each experimental series, data are presented as mean ± S.E.M. Statistical significance (P<0.05) for each variable was estimated by one-way analysis of variance (ANOVA) or t-test, where appropriate (SYSTAT, Inc., Evanston, IL, USA). A Dunnett’s test was used for post hoc analysis.

Results

General features of experimental animals

The diabetic animals (WKY-STZ and SHR-STZ) exhibited significantly lower body weights and higher serum glucose levels compared to the age-matched non-diabetic animals (WKY and SHR). Hypertensive animals (SHR and SHR-STZ) displayed elevated blood pressure, as expected. Although the absolute heart weights were comparable among all the groups, the heart/body weight ratio was significantly elevated in hypertensive, diabetic and diabetic hypertensive groups. SHR rats showed reduced liver size whereas SHR-STZ animals exhibited renal hypertrophy (Table 1).

Cell shortening and relengthening in cardiac myocytes

Sustained diabetes and hypertension, or both, did not affect cell length (CL). The peak shortening amplitude (PS) normalized to CL in left ventricular myocytes was not affected by hypertension alone but significantly enhanced by diabetes. Interestingly, interaction of diabetes and hypertension significantly reduced PS. Myocytes under sustained hypertension (SHR) demonstrated significantly prolonged time-to-90% relengthening (TR 90), associated with normal time-to-peak shortening (TPS), compared to its WKY counterparts. Sustained diabetes (WKY-STZ and SHR-STZ) significantly prolonged both TPS and TR 90 (Fig. 1). Lastly, the maximal velocities of shortening (+dL/dt) and relengthening (−dL/dt) were significantly reduced in both hypertensive groups (SHR and SHR-STZ). Diabetes alone exerted no effect on +dL/dt (Fig. 2). These data suggest that concomitant diabetes and hypertension is capable of eliciting an additive effect on certain (such as PS) but not all cardiac contractile parameters compared to either disease alone.
staircase in PS with increasing stimulating frequency. Neither diabetes nor hypertension alone induced any significant effect on the pattern of PS-frequency response. However, interaction of diabetes and hypertension demonstrated an additive effect by shifting the PS-frequency response curve to the left compared to that of WKY, indicating that the replenishment of intracellular Ca\(^{2+}\) storage may be significantly impaired under diabetic hypertension. Changes in the stimulating frequency from 0.1 Hz to 5 Hz did not affect the prolongation in TPS and TR\(_{90}\) by either disease alone or both (data not shown).

**Discussion**

This is the first study exhibiting that ventricular dysfunctions in diabetic hypertension stem from myopathy. Early hypertrophic responses in hyperglycemia, weight loss, discolored fur, reduced body weight gain, which is well characterized in both diabetes\(^5\) and hypertension.\(^{11}\) Overt cardiac mechanical abnormalities have been specially attributed to either diabetes or hypertensive (hypertrophic) cardiomyopathy and hypertensive (hypertrophic) cardiomyopathy, respectively. The two cardiomyopathies share certain similarities, especially diastolic dysfunctions manifested mainly as prolonged action potential duration and reduced rate of ventricular relaxation, at the levels of whole heart, papillary muscle\(^b\) and isolated ventricular myocytes,\(^5,7\) with the severity increasing with time.\(^5,7,10,28\) Sustained hypertension, unlike diabetes, often initiates cellular adaptive changes in the hearts including depressed sarcolemmal ion transport, reduced \(\beta\)-adrenergic activity and desensitized intracellular Ca\(^{2+}\) release,\(^8,28\) contributing to the onset of hypertrophic cardiomyopathy, which paradoxically exhibits significant similarities to diabetic cardiomyopathy.\(^10,11,28\) Changes in gene expression and contractile proteins are believed to play an important role in the pathogenesis of hypertrophic cardiomyopathy.\(^7\) Early hypertrophic responses in hypertension include a transient expression of proto-oncogene products that regulate cardiac growth and differentiation as well as a shift in the major form of contractile proteins such as from \(\alpha\)- to \(\beta\)-myosin heavy chain protein.\(^11\) Late hypertrophic responses such as marked increases in collagen and transforming growth factor-\(\beta1\) levels can often be detected, indicating that expression of specific extracellular matrix genes may contribute to fibrosis and impaired function.\(^11\) However, how these hypertrophic responses induce somewhat similar mechanical defects that are often seen in diabetes is essentially unknown.

Results from our current study revealed that myocyte shortening amplitude (PS) is unchanged in hypertension and enhanced in diabetes, consistent with our previous findings.\(^5,10,11\) The most important finding here is that diabetes and hypertension, synergistically depressed PS compared to either disease alone. This is consistent to findings at the multicellular level, and may be related to the interaction of diabetes and hypertension on cardiac excitation-contraction (E-C) coupling such as intracellular Ca\(^{2+}\) handling/contractile proteins. The

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Body wt (g)</th>
<th>Heart wt (g)</th>
<th>Heart wt/body wt (mg/g)</th>
<th>Liver wt/body wt (mg/g)</th>
<th>Kidney wt/body wt (mg/g)</th>
<th>Blood glucose (mg/dl)</th>
<th>Blood pressure (mmHg)</th>
</tr>
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<tbody>
<tr>
<td>WKY (6)</td>
<td>411 ± 12</td>
<td>1.54 ± 0.05</td>
<td>3.74 ± 0.07</td>
<td>32.8 ± 2.0</td>
<td>9.2 ± 0.24</td>
<td>110 ± 26</td>
<td>110 ± 3</td>
</tr>
<tr>
<td>SHR (6)</td>
<td>379 ± 14</td>
<td>1.57 ± 0.03</td>
<td>4.15 ± 0.21*</td>
<td>25.8 ± 0.5</td>
<td>6.4 ± 0.06</td>
<td>117 ± 20</td>
<td>180 ± 4*</td>
</tr>
<tr>
<td>WKY-STZ (4)</td>
<td>310 ± 29*</td>
<td>1.29 ± 0.17</td>
<td>4.16 ± 0.05*</td>
<td>35.1 ± 2.9</td>
<td>8.49 ± 1.12</td>
<td>348 ± 30*#</td>
<td>105 ± 1</td>
</tr>
<tr>
<td>SHR-STZ (4)</td>
<td>351 ± 27*</td>
<td>1.52 ± 0.15</td>
<td>4.32 ± 0.11*</td>
<td>35.1 ± 2.6#</td>
<td>9.12 ± 0.98*#</td>
<td>300 ± 42*#</td>
<td>186 ± 2*#</td>
</tr>
</tbody>
</table>

Mean ± S.E.M., *P<0.05 v WKY group, #P<0.05 v SHR group, number of animals is given in parentheses. Key: WKY, Wistar-Kyoto; SHR, spontaneously hypertensive; STZ, streptozotocin.
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Figure 1: Contractile properties of cardiac ventricular myocytes isolated from normal (WKY), hypertensive (SHR), diabetic (WKY-STZ) and diabetic hypertensive (SHR-STZ) rat hearts. Top panels: representative cell shortening traces from normotensive (top left: WKY and WKY-STZ) and hypertensive (top right: SHR and SHR-STZ) groups. The peak cell shortening (PS) was normalized to each other to better illustrate the time frame of cell shortening and relengthening. Middle panels: bar graphs depicting resting cell length (left panel) and peak shortening amplitude (right panel). Lower panels: time-to-peak shortening (TPS, left panel) and time-to-90% relengthening (TR90, right panel). Mean ± S.E.M., n = 64–78 cells/group, * P<0.05 v WKY group and # P<0.05 v SHR group.

PS-frequency relationship presented in Figure 4 also supported the notion of a synergistic response on PS in diabetic hypertensive cardiomyopathy. Furthermore, the maximal rate of shortening and relengthening (±dL/dt) often indicative of PS amplitude, also exhibited a trend of an additive response (normal in diabetes but depressed under hypertension and diabetic hypertension). The diminished myocyte contractile response to increased stimulating frequency in concomitant diabetes and hypertension, which does not exist in either disease alone, may indicate a much slower
replenishing process of the intracellular Ca\(^{2+}\) pool or significantly smaller pool size under diabetic hypertension. Nevertheless, the exact mechanism responsible for this synergism is largely unknown and deserves further investigation.

One of the obvious findings consistent with that observed at the multicellular level was prolonged duration of shortening (TPS) and relengthening (SERCA) in diabetes, hypertension or diabetic hypertension (although TPS was normal in hypertension). Several factors have been suggested to contribute to the prolonged duration of contraction and relaxation. Compromised cardiac contraction has been associated with a shift in myosin isozymes from the fast type (V\(_1\), α-MHC) to the slow type (V\(_3\), β-MHC) in diabetes.\(^{11}\) The myosin isozyme shift was reported to contribute to desensitization in Ca\(^{2+}\) sensitivity in cardiac myocytes.\(^{12}\) In the present study, the myocyte shortening in response to increases in extracellular Ca\(^{2+}\) concentration is clearly diminished in the WKY-STZ group compared to any other group. The normal Ca\(^{2+}\) responsiveness in SHR-STZ group may be a result of the compensated effect from hypertension, as the latter is known to enhance Ca\(^{2+}\) responsiveness. The prolonged duration of relaxation may be a result of impaired sarco(endo)plasm reticulum ATPase (SERCA) and/or other Ca\(^{2+}\) regulating proteins such as Na\(^+/\)Ca\(^{2+}\) exchange, which has been reported in diabetes, hypertension or diabetic hypertension.\(^{29,31}\) Several factors may be considered for the lack of additive/synergistic response on the duration of contraction and relaxation under concomitant diabetes and hypertension. Firstly, the cardiac Ca\(^{2+}\) regulating proteins such as SERCA and Na\(^+/\)Ca\(^{2+}\) exchange contribute predominantly to the duration of contraction and relaxation, whereas cardiac contractile proteins actin, myosin and titin determine predominantly the cross-linking machinery and therefore the con-

![Figure 2](image1.png)  

**Figure 2** The maximal velocity of shortening (+dL/dt, upper panel) and relengthening (−dL/dt, lower panel) of cardiac ventricular myocytes isolated from normal (WKY), hypertensive (SHR), diabetic (WKY-STZ) and diabetic hypertensive (SHR-STZ) rats. Mean ± S.E.M., n = 64–78 cells/group, *P < 0.05 v WKY group.

![Figure 3](image2.png)  

**Figure 3** Effect of increase in extracellular Ca\(^{2+}\) concentration (0.5–3 mM) on peak cell shortening (PS) in myocytes from normal (WKY), hypertensive (SHR), diabetic (WKY-STZ) and diabetic hypertensive (SHR-STZ) rats. Mean ± S.E.M., n = 13–16 cells/group, #P < 0.05 v WKY group.
Figure 4 Effects of stimulus frequency (0.1–5 Hz) on peak cell shortening (PS) in myocytes from normal (WKY), hypertensive (SHR), diabetic (WKY-STZ) and diabetic hypertensive (SHR-STZ) rat hearts. Each point represents peak shortening amplitude (PS) normalized to that of 0.1 Hz. The baseline PS at 0.1 Hz are: 12.5 ± 1.3%, 13.2 ± 1.3%, 13.4 ± 1.2%, and 9.8 ± 1.3%, in WKY, SHR, WKY-STZ and SHR-STZ groups, respectively. Mean ± S.E.M., # P<0.05 v WKY group.

In conclusion, our findings provide evidence that impaired cardiac contractile function in diabetic hypertension is likely due to changes at the single ventricular myocyte level, and concomitant diabetes and hypertension produced more severe cardiac mechanical defects than either disease alone (although in certain aspects). Given what we know about the role of cardiac contractile and Ca$^{2+}$ regulating proteins such as actin, myosin, titin, SERCA and Na$^+$/Ca$^{2+}$ exchange in cardiac excitation-contraction coupling, the direct impact of diabetic hypertension on the expression and function of these proteins would be intriguing to investigate.

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**References**


12. OHTA K, KIM S, IWAO H. Role of angiotensin-converting enzyme, adrenergic receptors, and blood