IGF-I attenuates diabetes-induced cardiac contractile dysfunction in ventricular myocytes

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Norby, Faye L., Loren E. Wold, Jinhong Duan, Kadon K. Hintz, and Jun Ren. IGF-I attenuates diabetes-induced cardiac contractile dysfunction in ventricular myocytes. Am J Physiol Endocrinol Metab 283: E658–E666, 2002.—Diabetic cardiomyopathy is characterized by impaired ventricular contraction and altered function of insulin-like growth factor I (IGF-I), a key factor for cardiac growth and function. Endogenous IGF-I has been shown to alleviate diabetic cardiomyopathy. This study was designed to evaluate exogenous IGF-I treatment on the development of diabetic cardiomyopathy. Adult rats were divided into four groups: control, control + IGF-I, diabetic, and diabetic + IGF-I. Streptozotocin (STZ; 55 mg/kg) was used to induce experimental diabetes immediately followed by a 7-wk IGF-I (3 mg·kg⁻¹·day⁻¹ ip) treatment. Mechanical properties were assessed in ventricular myocytes including peak shortening (PS), time-to-PS (TPS), time-to-90% relengthening (TR90), and maximal velocity of shortening/relengthening (±dL/dt). Intracellular Ca²⁺ transients were evaluated as Ca²⁺-induced Ca²⁺ release and Ca²⁺ clearing constant. Levels of sarco(endo)plasmic reticulum Ca²⁺-ATPase (SERCA), phospholamban (PLB), and glucose transporter (GLUT4) were assessed by Western blot. STZ caused significant weight loss and elevated blood glucose, demonstrating the diabetic status. The diabetic state is associated with reduced serum IGF-I levels, which were restored by IGF-I treatment. Diabetic myocytes showed reduced PS and ±dL/dt as well as prolonged TPS, TR90, and intracellular Ca²⁺ clearing compared with control. IGF-I treatment prevented the diabetes-induced abnormalities in PS, ±dL/dt, TR90, and Ca²⁺ clearing but not TPS. The levels of SERCA and GLUT4, but not PLB, were significantly reduced in diabetic hearts compared with controls. IGF-I treatment restored the diabetes-induced decline in SERCA, whereas it had no effect on GLUT4 and PLB levels. These results suggest that exogenous IGF-I treatment may ameliorate contractile disturbances in cardiomyocytes from diabetic animals and could provide therapeutic potential in the treatment of diabetic cardiomyopathy.

Diabetic cardiomyopathy; insulin-like growth factor I; ventricular myocytes; sarcoplasmic reticulum Ca²⁺-ATPase; glucose transporter 4

Diabetes is a major risk factor leading to increased cardiovascular mortality and morbidity (19, 40). Accumulating evidence has demonstrated the existence of a specific cardiomyopathy associated with diabetes. The myopathy occurs independently of micro/macrovacular changes and contributes significantly to death in the diabetic population (17, 19). Diastolic dysfunction is the most prominent defect of diabetic cardiomyopathy, manifested by decreased compliance, prolonged myocardial relaxation, and altered intracellular Ca²⁺ homeostasis (17, 32, 33). Recent studies suggest that diabetic cardiomyopathy may be directly due to impaired mechanical function at the single ventricular myocyte level in both chemically induced and genetically predisposed experimental diabetic models (32, 33, 36).

Insulin-like growth factor I (IGF-I) is an important growth factor for cell differentiation and proliferation (12, 35, 38). It is produced in the heart as well as other tissues and acts as both an autocrine and a paracrine hormone. Its main function in the heart is to stimulate cardiac growth and contractile function (10, 35). IGF-I has been shown to stimulate protein synthesis, increase inositol 1,4,5-trisphosphate levels, activate tyrosine kinase, tyrosine kinase phosphate, phosphatidylinositol 3-kinase, and protein kinase C, and enhance myocardial contraction and intracellular Ca²⁺ sensitivity (9, 15, 35, 41). Approximately 95% of circulating IGF-I is bound to IGF-I-binding proteins (IGFBPs), predominantly IGFBP-3, which are believed to modulate the actions of IGF-I in a cellular-specific manner (12). Although free IGF-I has a relatively short half-life (~15 min), factors like IGFBPs may extend the actual half-life of IGF-I in the body to ~10 h (7). IGF-I and IGFBP levels are reduced in diabetes, possibly as a result of insufficient portal insulinization and hepatic growth hormone resistance (3, 5). The reduced IGF-I and IGFBP levels in diabetes raise the possibility that this pathway may contribute to the pathogenesis of diabetic cardiomyopathy. Clinically, IGF-I has been used as a replacement for insulin to facilitate glucose transport and metabolism in diabetes, especially under insulin-resistant conditions (1, 27). Combined treatment with IGF-I and insulin in diabetic patients was reported to correct growth hormone hy-
persecution, improve insulin sensitivity and glucose control, and decrease the requirement for insulin (39). Our laboratory has recently shown that diabetic cardiomyopathy may be prevented with insulin-sensitizing antidiabetics such as metformin (34). Others have shown that cardiac overexpression of IGF-I inhibits the development of diabetic cardiomyopathy (23). Exogenously administered IGF-I has been shown to have both positive (16) and negative (31) effects on the development of cardiac disorders such as heart failure. However, the impact of exogenous IGF-I administration on diabetes-associated cardiac contractile dysfunction is largely unknown.

Although transgenic overexpression of IGF-I reverses diabetic cardiomyopathy in mice (23), it has not been used clinically for this purpose. To evaluate the therapeutic potential of exogenous IGF-I administration in diabetic cardiomyopathy, we determined the impact of exogenous IGF-I administration on diabetes-induced impairment in cardiac excitation-contraction (E-C) coupling by using state-of-the-art video-based edge detection and intracellular Ca\(^{2+}\) recording in isolated ventricular myocytes. Because mechanical dysfunction that characterizes diabetic cardiomyopathy is associated with changes in intracellular Ca\(^{2+}\) regulation and glucose transport (13, 18, 26, 28, 30), the impact of IGF-I treatment on the abundance of sarco-(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA), phospholamban (PLB), and GLUT4 was assessed by Western blotting.

MATERIALS AND METHODS

Experimental animals and assay for IGF-I and glucose. All animal experimentation was conducted in accordance with humane animal care standards outlined in the National Institutes of Health Guide for the Care and Use of Experimental Animals and approved by the Animal Investigation Committee of the University of North Dakota School of Medicine. Briefly, 6-wk-old male Sprague-Dawley (SD) rats (Harlan Sprague Dawley, Indianapolis, IN) were made diabetic with a single intravenous tail vein injection of streptozotocin (STZ; 55 mg/kg iv), as described (33), and maintained for 7 wk of untreated diabetes. A group of vehicle-treated, age-matched euglycemic rats was selected in parallel with the STZ-treated rats. IGF-I (Genentech, San Francisco, CA, and National Hormone and Peptide Program, National Institute of Diabetes and Digestive and Kidney Diseases) was supplied for 7 wk (3 mg·kg\(^{-1}\)·day\(^{-1}\) ip). Saline was used in the non-IGF-I treatment groups. The diabetic state was as assessed by weekly measurement of the glucose level by means of an Accu-ChekII glucometer (model 792, Boehringer Mannheim Diagnostics, Indianapolis, IN). At the time animals were killed, plasma was collected, and plasma total IGF-I concentration, single-emission photomultiplier system (IonOptix) in phospholamban (PLB), and GLUT4 was assessed by Western blotting.

Intracellular Ca\(^{2+}\) transient measurement. Intracellular fluorescence properties were measured using a dual-excitation, single-emission photomultiplier system (IonOptix) in myocytes loaded with fura 2-AM (0.5 μM). Myocytes were placed on an inverted microscope and imaged through an Olympus (model: IX-70) Fluor ×40 oil objective. Myocytes were exposed to light emitted by a 75-W halogen lamp through either a 360- or 380-nm filter while being stimulated to contract at 0.5 Hz. Fluorescence emissions were detected between 480 and 520 nm by a photomultiplier tube after the 360-nm excitation reading was repeated at the end of the protocol. Qualitative evaluation of the electrically stimulated Ca\(^{2+}\)-induced Ca\(^{2+}\) release was inferred from the fura fluorescence intensity changes. A Chebyshev curve-fitting technique was used to evaluate the intracellular Ca\(^{2+}\) clearing or decay kinetics to calculate the intracellular Ca\(^{2+}\) decay constant (τ, the duration for 2/3 Ca\(^{2+}\) transient decay from peak). The goodness of fit was confirmed by visually inspecting the fitted curve to the actual data. Deviations were not accepted (32). Myocyte shortening was also evaluated in a cohort of the fura 2-loaded ventricular myocytes simultaneously to compare their temporal relationship with the fluorescence signal. However, their mechanical properties (although quite similar to those from the nonloaded cells) were not used for data summary due to the Ca\(^{2+}\) buffering effect of fura 2 (44).
Western analysis of SERCA2, PLB, and GLUT4. Membrane proteins from the left ventricular myocardium of each heart were isolated as described (18). Freshly dissected hearts were homogenized and centrifuged at 1,000 g for 10 min. The supernatants were centrifuged at 70,000 g for 30 min at 4°C. The 100,000-g pellets were cellular membrane fractions and were used for immunoblotting of SERCA2, PLB, and GLUT4. We confirmed that these membrane fractions did not contain any detectable collagens. Membrane proteins (50 μg/lane) were separated on 7% (SERCA2a) or 15% (PLB and GLUT4) SDS-polyacrylamide gels in a minigel apparatus (Mini-PROTEAN II, Bio-Rad) and transferred to polyvinylidene difluoride membranes. The membranes were blocked (4% Block Ace, Dainippon Pharmaceutical, Osaka, Japan) and then incubated with anti-SERCA2 (1:1,000 dilution), anti-PLB (1:1,000), and anti-GLUT4 (1:4,000) antibodies [monoclonal antibodies to SERCA2a (A7R5) and PLB (2D12) were kindly provided by Dr. Larry Jones, Indiana University School of Medicine]. GLUT4 monoclonal antibody (2D12) were kindly provided by Dr. Larry Jones, Indiana University School of Medicine]. GLUT4 monoclonal antibody (2D12) was obtained from Chemicon International (Temecula, CA). The antigens were detected by the enhanced luminescence method (ECL Western blotting detection kit, Amersham) with peroxidase-linked anti-mouse (SERCA2a and PLB) or anti-rabbit (GLUT4) IgG (1:5,000 dilution). After immunoblotting, the film was scanned, and the intensity of immunoblot bands was detected with a Bio-Rad calibrated densitometer (model GS-800).

Statistical analyses. For each experimental series, data are presented as means ± SE. Statistical significance (P < 0.05) for each variable was estimated by analysis of variance (ANOVA) or t-test, where appropriate (SYSTAT, Evanston, IL). A Dunnett’s test was used for post hoc analysis.

RESULTS

General features of experimental animals. Chronic diabetes induced severe deficiency in serum IGF-I levels, which was restored by exogenous IGF-I administration. The diabetic animals exhibited significantly reduced body weight gain and higher blood glucose levels compared with their age-matched nondiabetic controls. Diabetic animals displayed decreased heart weight; however, the liver and kidney weights were not significantly affected compared with the age-matched controls. Exogenous IGF-I administration did not significantly affect the biometric indexes in either diabetic or control groups (Table 1).

Cell shortening and relengthening in cardiac myocytes. Peak shortening (PS) amplitude normalized to cell length was significantly decreased in ventricular myocytes under STZ-induced diabetes. Myocytes from the diabetic group also demonstrated significantly prolonged time-to-peak shortening (TPS) and time-to-90% relengthening (TR90) compared with control, consistent with our previous findings (33, 34). IGF-I administration completely abolished the diabetes-induced abnormalities of PS and TR90 but did not affect TPS. None of these mechanical indexes (PS, TPS, TR90) was affected by IGF-I administration in the control group (Fig. 1, A-D). The maximal velocities of shortening (+dL/dt) and relengthening (−dL/dt) were significantly reduced by diabetes; however, IGF-I partially restored the diabetes-induced decrease in ±dL/dt (Fig. 2, A and B). In addition, IGF-I administration did not affect +dL/dt but attenuated −dL/dt in the control group.

Effect of IGF-I administration on intracellular Ca2+ transients. To evaluate whether the IGF-I-induced beneficial effects on diabetic cardiomyopathy were due to its effect on intracellular Ca2+ homeostasis, intracellular Ca2+ transients were evaluated in fura 2-loaded myocytes. Myocytes from diabetic animals showed reduced baseline Ca2+ and electrically stimulated Ca2+-induced Ca2+ release levels compared with those from the control group. Intracellular Ca2+ release levels in the control group (data not shown). These data may indicate a slowed Ca2+ extrusion rate. Consistent with its effect on TR90, IGF-I administration ablated the diabetes-induced abnormal intracellular Ca2+ clearing. Finally, IGF-I administration itself decreased resting intracellular Ca2+ levels in the control group (Fig. 3, A–C).

Effect of extracellular Ca2+ on myocyte shortening. To examine the influence of extracellular Ca2+ levels on PS amplitude, myocytes were first stimulated to contract at 0.5 Hz in a contractile buffer defined in MATERIALS AND METHODS with 0.5 mM CaCl2. After the steady-state PS had been recorded, the extracellular Ca2+ level was increased to 1.0, 2.0, and 3.0 mM, with a 5-min interval between each concentration. Figure 4 shows that the PS amplitude was significantly depressed in the diabetic myocytes under 0.5 mM extracellular Ca2+ concentration, consistent with the results obtained at 1.0 mM extracellular Ca2+ (Fig. 1). Increases in extracellular Ca2+ concentration from 0.5 to 3 mM resulted in a positive staircase (although not at 3 mM) in PS amplitude in myocytes from the control group. Diabetes significantly shifted the Ca2+ concentration-response curve downward, which was partially prevented by IGF-I administration. IGF-I administration did not significantly affect the Ca2+ response curve in the control group (data not shown). These data may suggest a possible alteration in myocyte responsiveness to Ca2+ in diabetes, which has been reported.

Table 1. General features of experimental animals

<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, g</th>
<th>Kidney Wt, g</th>
<th>Blood Glucose, mg/dl</th>
<th>Serum IGF-I, ng/ml</th>
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<tbody>
<tr>
<td>Control (9)</td>
<td>434 ± 21</td>
<td>1.85 ± 0.07</td>
<td>4.33 ± 0.25</td>
<td>14.2 ± 0.5</td>
<td>3.16 ± 0.17</td>
<td>94 ± 4</td>
<td>711 ± 143</td>
</tr>
<tr>
<td>Diabetic (10)</td>
<td>293 ± 60*</td>
<td>1.37 ± 0.17*</td>
<td>5.23 ± 0.53</td>
<td>12.5 ± 1.7</td>
<td>3.35 ± 0.50</td>
<td>333 ± 42*</td>
<td>252 ± 81*</td>
</tr>
<tr>
<td>Control + IGF-I (9)</td>
<td>451 ± 14*</td>
<td>1.78 ± 0.05</td>
<td>3.96 ± 0.15</td>
<td>12.7 ± 2.7</td>
<td>3.09 ± 0.15</td>
<td>87 ± 8</td>
<td>824 ± 59</td>
</tr>
<tr>
<td>Diabetic + IGF-I (10)</td>
<td>280 ± 63*</td>
<td>1.25 ± 0.17*</td>
<td>5.12 ± 0.61</td>
<td>12.8 ± 1.9</td>
<td>3.03 ± 0.53</td>
<td>318 ± 42*</td>
<td>504 ± 115†</td>
</tr>
</tbody>
</table>

Values are means ± SE; nos. of animals per group are given in parentheses. *P < 0.05 vs. control group, †P < 0.05 vs. diabetic group.
previously in both chemically induced and genetically predisposed diabetic models (22, 32).

Effect of stimulation frequency on myocyte shortening. Rat hearts normally contract at very high frequencies (300 beats/min or 5 Hz), whereas our baseline studies were conducted at 0.5 Hz. To assess for possible derangement of cardiac E-C coupling at higher frequencies, we increased the stimulating frequency up to 5 Hz and recorded steady-state peak shortening. Cells were initially stimulated to contract at 0.5 Hz for 5 min to ensure steady state before the frequency study commenced. All recordings were normalized to PS at 0.1 Hz of the same myocyte. Figure 5 shows a negative staircase in PS with increasing stimulation frequency. The pattern of response was comparable between the diabetic and control groups. However, myocytes from the control/IGF-I group displayed a significantly higher rate of decline in PS when stimulatory frequency was increased from 0.1 to 0.5 Hz (PS at 0.1 Hz was normalized to 100%). Changes in the stimulating frequency from 0.1 to 5 Hz did not affect the prolongation in either TPS or TR90 in diabetic rats (data not shown).

Western blotting of SERCA2, PLB, and GLUT4. The protein levels of SERCA2, PLB, and GLUT4 from hearts of control and diabetic rats with or without IGF-I treatment are shown in Fig. 6. Consistent with previous reports (13, 18, 26), both SERCA2 and GLUT4 protein levels were significantly reduced, whereas PLB was unchanged in STZ-induced diabetic hearts compared with the control group. IGF-I supplementation did not affect GLUT4 and PLB levels in either control or diabetic group. Interestingly, the diabetes-induced decline in SERCA2 level was restored by the 7-wk IGF-I treatment. IGF-I supplementation also significantly enhanced the SERCA2 level in the control hearts. These observations are consistent with the functional data of improved intracellular Ca2+ clearance and duration of relengthening (TR90) with IGF-I treatment.

DISCUSSION

The major findings from our study demonstrated that exogenous IGF-I treatment inhibits the development of certain characteristics of diabetic cardiomyopathy such as abnormal E-C coupling in isolated ventricular myocytes. Our results revealed decreased myocyte PS, reduced ±dL/dt, and markedly prolonged duration of shortening and relengthening in myocytes from STZ-induced diabetic hearts. These mechanical abnormalities may be underscored by altered intracellular Ca2+ homeostasis, shown as slowed intracellular Ca2+ removal, reduced resting intracellular Ca2+ levels, and Ca2+-induced Ca2+ release. Our immunostaining study indicated that the impaired intracellular
Ca$^{2+}$ homeostasis may be associated with a reduction in the main Ca$^{2+}$-regulating protein SERCA and/or indirectly with metabolic derangement due to the compromised membrane glucose transporter GLUT4 under the diabetic state, consistent with earlier observations (13, 18, 26, 28). Interestingly, these diabetes-induced cardiac mechanical dysfunctions (except prolonged TPS) were significantly inhibited by daily exogenous IGF-I treatment, supporting a recent report using endogenous transgenic overexpression of IGF-I (23). Our study also indicated that IGF-I treatment may protect against a diabetes-induced decline in cardiac SERCA2 but not GLUT4 protein abundance. These observations make IGF-I of potential interest for the treatment of diabetes-induced cardiac myopathic complications.

In this study, STZ-treated rats developed typical signs of diabetes, such as hyperglycemia and growth retardation associated with severely reduced plasma IGF-I levels. The hyperglycemic status in STZ rats was not affected by exogenous IGF-I treatment, which is in line with the lack of effect of IGF-I on GLUT4 levels. Reduction in GLUT4 in the diabetic heart is likely due to hyperglycemia as opposed to lack of insulin (13, 18). A recent study found that mice with cardiac-specific knockout of the insulin receptor actually have increased cardiac GLUT4 content (2). The metabolic efficacy of IGF-I is only 6–10% of that of insulin (6, 20). Although administration of IGF-I was shown to reduce blood glucose (27, 46), conflicting observations have
also been reported (15, 23, 42). The inconsistency in the glucose metabolic properties of IGF-I may be related to the manner of administration, timing of IGF-I injection, and glucose measurement. Data from our study confirmed the previous findings that sustained diabetes leads to severe loss of IGF-I (3, 5), possibly due to insufficient portal insulinization and hepatic growth hormone resistance in diabetes. Interestingly, our exogenous IGF-I administration regimen was able to restore >70% of the IGF-I under the diabetic condition. Because the dose of IGF-I administration (3 mg·kg⁻¹·day⁻¹) is relatively high (15, 16), it may be speculated that the diabetes-induced decrease in the level of IGFBPs may not be able to provide enough "reservoir" capacity, even though they may be "upregulated" by IGF-I administration (42).

The present study revealed decreased PS and ±dL/dt, associated with prolonged TPS and TR₉₀ in myocytes from diabetic hearts, consistent with the hallmarks of diabetic cardiomyopathy reported previously in whole heart (11), papillary muscle (36), and isolated myocytes (32, 33, 44). Our present observation of a reduced intracellular Ca²⁺ transient decay is consistent with the prolonged TR₉₀ and may be a result of impaired SERCA and/or Na⁺/Ca²⁺ exchange in diabetes, consistent with our immunostaining result and earlier reports (8, 26). On the other hand, the reduced resting Ca²⁺ level and Ca²⁺-induced Ca²⁺ release, which correlate with reduced PS and ±dL/dt, may have resulted from diabetes-induced reduction in membrane Ca²⁺ channel conduction and sarcoplasmic reticulum Ca²⁺ load (8, 30, 43). In addition, several other factors have also been speculated to contribute to the diabetes-induced cardiac mechanical dysfunctions such as alterations in Ca²⁺-calmodulin-dependent protein kinase (30), myosin isozyme switch (from V₁ to V₃) (14), and myofilament Ca²⁺ sensitivity (22, 32). Changes in the contractile apparatus would affect not only cardiac contractility (PS) but also the velocity of contraction (+dL/dt), which may subsequently manipulate the duration of contraction (TPS). Myosin isozyme switch has been shown to lead directly to a decrease in Ca²⁺ responsiveness (29) and may be responsible for the depressed myofilament Ca²⁺ sensitivity in diabetes (22, 25). This is supported by the observation from the present study that myocyte shortening in response to increased extracellular Ca²⁺ concentration (0.5–3 mM) is clearly diminished in the diabetic group compared with the control group. Because SERCA contributes to ~92% of the cytosolic Ca²⁺ removal workload in rat hearts (4), our finding of an overt reduction in SERCA2a protein level in STZ-induced diabetic hearts should have provided one of the most compelling explanations for the slowed intracellular Ca²⁺ clearing and prolonged duration of relaxation (TR₉₀). PLB is the main inhibitor of SERCA, acting to keep SERCA function "in check." An increase in the PLB-to-SERCA2 ratio reduces the SERCA Ca²⁺ affinity and activity, leading to prolonged relaxation and a reduced contractility. On the other hand, a decreased PLB-to-SERCA2 ratio improves the cardiac Ca²⁺ cycling and pumping function (28). Our results clearly show an enhanced PLB-to-SERCA2 ratio in diabetic hearts, consistent with the prolonged TR₉₀ and reduced PS. More importantly, results from our study indicated that exogenous IGF-I treatment prevented the slowed intracellular Ca²⁺ clearing, which may underscore its protective effect on prolonged relaxation (TR₉₀) in diabetes. This was nicely associated with the ability of exogenous IGF-I treatment to restore the PLB-to-SERCA2 ratio to the control level, providing a plausible explanation for its ability to prevent diabetes-induced defects in PS, τ, and TR₉₀. The significance of the PLB-to-SERCA2 ratio on

Fig. 4. Effect of increased extracellular Ca²⁺ concentration (0.5–3 mM) on PS. Values are means ± SE; n = 15–31 cells evenly distributed from 5–9 rats per group. *P < 0.05 vs. control group, #P < 0.05 vs. diabetic group.

Fig. 5. Effects of increased stimulus frequency (0.1–5 Hz) on PS in myocytes from control and diabetic rats with or without IGF-I administration. Each point represents PS amplitude normalized to that of 0.1 Hz. Values are means ± SE; n = 25 cells evenly distributed from 5–7 rats per group. #P < 0.05 vs. control group.
myocardial contractile regulation has been demonstrated in rodent models with variable expression levels of PLB and SERCA (28).

In our study, certain diabetes-induced mechanical defects were not improved or protected by exogenous IGF-I treatment. For example, IGF-I improved the diabetes-induced reduction in PS but not Ca2⁺/H11001-induced Ca2⁺/H11001 release. Although the underlying mechanism is largely unknown, the ability of IGF-I to enhance myofilament Ca2⁺ sensitivity (9) may play a role. This is somewhat supported by the results shown in Fig. 4, where myocytes from the diabetic + IGF-I group exhibit an improved PS at low (<1 mM) extracellular Ca2⁺ levels compared with the myocytes from the diabetic group. However, an additional increase in extracellular Ca2⁺ level (to 3 mM) failed to induce a further increase in PS within the diabetic + IGF-I group. The mechanism of action is unknown, although a potential "saturating" effect of IGF-I on myofilament Ca2⁺ sensitivity may be speculated. In addition, potential involvement of Ca2⁺-independent mechanisms should not be excluded at this time. Second, results from this study revealed that exogenous IGF-I administration lowered the resting intracellular Ca2⁺ levels in the nondiabetic group but did not affect the already reduced resting intracellular Ca2⁺ levels in myocytes from the diabetic group. This IGF-I-induced reduction in resting intracellular Ca2⁺ level may be associated with an enhanced SERCA Ca2⁺ clearing ability in the control + IGF-I group (Fig. 6) and is consistent with the vasodilatory and cardioprotective effect against Ca2⁺ overload under pathological conditions such as heart failure (15, 38). Third, the frequency-PS relationship was not affected by diabetes (Fig. 5), indicating a preserved sarcoplasmic reticulum (SR)-replenishing function in diabetic hearts. However, IGF-I treatment caused a steeper decline of the frequency-PS curve in the control group but not in the diabetic group. One possible explanation is that IGF-I may significantly augment the basal SR Ca2⁺ load (at 0.1 Hz) in the control group, which may be offset by diabetes (30). It may also reflect an adverse effect of IGF-I treatment. Further study is warranted to determine the effect of IGF-I on SR Ca2⁺ load and replenishing capacity. Last, but not least, the lack of effect of IGF-I treatment on TPS indicates that IGF-I may not have any significant effect on the key rate-limiting components determining the length of contraction duration such as SR Ca2⁺ release, troponin, tropomyosin, and actin-myosin cross-bridge linking.

Using the IGF-I transgene technique, Anversa’s group (Kajstura et al. (23) and Kerr et al. (24)) recently showed that cardiac-specific IGF-I overexpression not only improves cardiac contractile function but also offers protection against oxidative stress, apoptosis, and, ultimately, diabetic cardiomyopathy. Our present study revealed beneficial effects of IGF-I even in the presence of persistent hyperglycemia, thus excluding any role of glucose metabolism in IGF-I-induced cardiac protection. IGF-I is ~10 times more potent than insulin in modulating the cardiac function (9, 36) and is capable of improving the sensitivity of insulin (35), which is severely deficient in STZ-induced diabetes (21). These inotropic properties of IGF-I may have provided the therapeutic advantage of IGF-I over insulin (or their use in combination) in the management of compromised heart function under diabetes. Another point worth mentioning is that IGF-I adminis-
tration may enhance sympathetic catecholamine release, thus improving cardiac function (15). However, this is not likely to be the scenario for enhanced ventricular myocyte mechanics under our in vitro condition, because limited catecholamines would be available. Our present study adds to the growing body of data supporting the prophylactic and interventional use of IGF-I treatment for cardiac complications such as diabetic cardiomyopathy and congestive heart failure via direct beneficial actions on survival and function of ventricular myocytes (35, 38). However, caution must be used when interpreting data from our current cellular study, since in vivo cardiac function evaluation (such as echocardiograph) was not provided.

Although accumulating evidence, including the present study, has shown a beneficial effect of IGF-I on cardiac function, the use of exogenous IGF-I treatment in cardiovascular diseases may be hampered by the systemic adverse effects associated with IGF-I, ranging from dizziness, fatigue, palpitations, and flushing to dyspnea or even transient cerebral dysfunction (37). These events often occur during acute intravenous infusion with a high dose of IGF-I. On the other hand, due to the growth-stimulating properties of IGF-I, extremely prolonged use may also be associated with unwanted growth promotion such as atherosclerosis. Administration of exogenous IGF-I has been shown to stimulate an increase in circulating IGFBP levels, although this increase may be insufficient to alter the IGF-I binding capacity (45). However, the potential alteration of IGFBP levels due to exogenous IGF-I administration, which was not examined in our study, may be an important drawback for the exogenous IGF-I treatment, since IGFBPs are known to affect the biologically active fraction of IGF-I, or free IGF-I. Further understanding of the specific cardiac pathways modulated by IGF-I and IGFBPs may help us design a useful treatment regimen for diabetic cardiomyopathy and other cardiac complications.

In conclusion, our findings provide evidence that impaired cardiac E-C coupling in diabetes is significantly improved by exogenous IGF-I administration, probably by improved intracellular Ca\(^{2+}\) homeostasis and Ca\(^{2+}\)-regulating protein function such as SERCA. Given what we know about the ability of IGF-I to promote cell survival and cardiac performance (35, 38), the in-depth mechanism of action and clinical value of employing IGF-I in the prevention and treatment of diabetic cardiomyopathy warrant further investigation.

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REFERENCES


23. **Kajstura J, Fiordaliso F, Andreoli AM, Li B, Chimenti S, E666 IGF-I AND DIABETIC HEART**


