Attenuated cardiac contractile responsiveness to insulin-like growth factor I in ventricular myocytes from biobreeding spontaneous diabetic rats

Jun Ren*

Department of Pharmacology, Physiology, and Therapeutics, University of North Dakota School of Medicine, 501 N. Columbia Road, Grand Forks, ND 58203, USA

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Abstract

Objective: Insulin-like growth factor I (IGF-1) stimulates cardiac growth and contraction, but resistance to its action has been reported in diabetes. This study was to determine if IGF-1-induced cardiac contractile action is altered in rats genetically predisposed to diabetes.

Method: Ventricular myocytes were isolated from spontaneously biobreeding diabetes-prone (BB/DP) rats and their diabetes-resistant littermates (BB/DR). Mechanical properties were evaluated in cardiomyocytes using a video-based edge-detection system. Myocytes were electrically stimulated at 0.5 Hz. Contractile properties analyzed included peak shortening (PS), time-to-PS (TPS) and time-to-90% relengthening (TR90). Intracellular Ca transients were measured as changes in fura-2 fluorescence intensity (ΔFFI).

Results: Myocytes from BB/DP rats displayed increased PS, prolonged TPS and TR90, as well as reduced resting FFI compared to the BB/DR group. IGF-1 (10⁻¹⁰–10⁻⁶ M) caused a dose-dependent increase in PS in myocytes from BB/DR but not BB/DP rats. The increase of PS was blunted by IGF-1 antagonist H-1356, phosphatidylinositol-3 (PI-3) kinase inhibitor wortmannin, but not tyrosine kinase inhibitor genistein. None of these agents affected responses to IGF-1 in BB/DP myocytes. Interestingly, IGF-1 elicited a comparable dose-dependent increase in Ca²⁺ transients in myocytes from both BB/DR and BB/DP rats.

Conclusion: These results suggest that the attenuation of IGF-1-induced cardiac contractile response in chemically-induced diabetes also exists in diabetes of genetic origin, possibly due to mechanisms involving PI-3 kinase and intracellular Ca²⁺ sensitivity. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Calcium (cellular); Contractile function; Diabetes; Excitation–contraction (E–C) coupling; Growth factors; Myocytes

1. Introduction

Clinical and experimental evidence has indicated that there is an increased cardiovascular mortality in diabetes mellitus, both insulin-dependent and non-insulin dependent. The mechanisms behind this high incidence remain largely obscure but may be associated with macro/micro-angiopathy, autonomic nephropathy and other factors leading to structural, metabolic or functional alterations of the heart [1–3]. A specific form of cardiac muscle disease during development of diabetes, namely diabetic cardiomyopathy, has also been recognized as an independent causal factor for the enhanced mortality and morbidity in diabetes [4,5]. Impaired myocardial contraction has been shown to be the most prominent mechanical defect associated with diabetic cardiomyopathy and is characterized by prolonged contraction duration, reduced force and decreased compliance [5,6]. The etiology of diabetic cardiomyopathy is complex and may involve metabolic derangements, decreased autonomic function, and abnormalities in various hormones or proteins that regulate intracellular ion homeostasis, particular Ca²⁺ [7]. Recently, focus has been geared towards impaired insulin sensitivity and glucose metabolism in the pathogenesis of diabetic cardiomyopathy. Elevated glucose may directly contribute to the development of diabetic cardiomyopathy, which can be protected by insulin sensitizing agents such as thiazolidinediones and metformin [6,8,9]. These observations suggest a link between insulin sensitivity, glucose control and diabetic cardiomyopathy.

Insulin-like growth factor I (IGF-1) is very similar to
insulin in structure and cellular action and can be used as a replacement for insulin to control blood glucose in diabetes, especially when there is insulin resistance. IGF-1 is expressed and synthesized by various cell types and exerts its actions, such as enhancing cardiac growth and myocardial contraction, in a paracrine/autocrine fashion. Although long-term administration of IGF-1 can be associated with cardiac hypertrophy and other cardiovascular complications, IGF-1 has been shown to improve hemodynamics, myocardial function, energy metabolism and clinical status. We recently observed resistance to IGF-1 in myocardial contraction in streptozotocin (STZ)-induced diabetes [10]. An altered cardiac IGF-1/IGF-1 receptor level has also been reported in diabetic patients and experimental animals, suggesting a role for IGF-1 in diabetic cardiomyopathy [11,12].

The aim of the present investigation was to examine if changes of IGF-1-induced cardiac contractile function also persist in rats genetically predisposed to diabetes. The spontaneously diabetic BB Wistar rat was used as the model, which displays a diabetic syndrome, consisting of hypoinsulinemia, hyperglycemia and glycosuria. It is a result of cell-mediated autoimmune destruction of pancreatic β-cells, a process that leads to insulin deficiency and subsequently an increase in blood glucose and free fatty acids. As a model of diabetes that depends on insulin for survival, the BB Wistar rat is the closest counterpart to human type I diabetes. Distinct morphological and functional abnormalities have been reported in BB Wistar rat myocardium, including loss of myofilaments, disruption of mitochondria, dilation of sarcoplasmic reticulum, depressed myocardial contractility, rate of ventricular relaxation and sarcoplasmic reticulum Ca$^{2+}$ [13–16]. These effects are somewhat similar to those reported in chemically-induced diabetic hearts [5,6,10].

2. Methods

2.1. Animals

The investigation conforms with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Male biobreeding diabetes-prone (BB/DP) and age-matched diabetes-resistant (BB/DR) rats were purchased at 60 days of age from the breeding colony at the University of Massachusetts, Biomedical Research Models. The rats were housed individually and allowed free access to standard laboratory rat chow and tap water. Upon the onset of type I diabetes, indicated by glucosuria, the BB/DP rats were anesthetized with brevital sodium (50 mg/kg, i.p.) and a sustained-release insulin implant (Linplant, Linshin, Canada) was inserted in the dorsal neck region of each animal. The length of the Linplant was adjusted to maintain chronic moderate hyperglycemia in the BB/DP rats. Blood glucose levels were measured weekly using a glucose monitor (Accu-ChekII, model 792, Boehringer Mannheim Diagnostics, Indianapolis, IN, USA). The active life of the Linplant was approximately 40 days, thereby eliminating the need for daily insulin injection. Re-implantation was performed when blood glucose levels exceeded 20 mM. The animals were sacrificed at 1 year of age.

2.2. Glucose tolerance test

Both BB/DR and BB/DP rats were fasted for 12 h and then given an intraperitoneal (i.p.) injection of glucose (2 g/kg body wt.). Blood samples were drawn from the tail 15 min and immediately before the glucose challenge and 15, 60 and 120 min thereafter. Serum glucose levels were determined using the Accu-Chek II glucose analyzer described above.

2.3. Cell isolation procedures

Single ventricular myocytes were enzymatically isolated [10]. Briefly, hearts were rapidly removed and perfused (at 37°C) with Krebs±Henseleit bicarbonate (KHB) buffer containing (in mM): 118 NaCl, 4.7 KCl, 1.25 CaCl$_2$, 1.2 MgSO$_4$, 1.2 KH$_2$PO$_4$, 25 NaHCO$_3$, 10 N-(2-hydroxy-ethyl)-piperazine-N$'$(2-ethanesulfonic acid) (Hepes) and 11.1 glucose. The KHB was equilibrated with 5%CO$_2$–95% O$_2$. Hearts were subsequently perfused with a nominally Ca$^{2+}$-free KHB buffer for 2–3 min until spontaneous contractions ceased. This was followed by a 20-min perfusion with Ca$^{2+}$-free KHB containing 223 U/ml collagenase (Worthington Biochemical, Freehold, NJ, USA) and 0.1 mg/ml hyaluronidase (Sigma, St. Louis, MO, USA). After perfusion, ventricles were removed and minced, under sterile conditions, and incubated with the above enzymatic 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 enzymatic solution by centrifugation (60 g for 30 s). Myocytes were resuspended in a sterile filtered, Ca$^{2+}$-free Tyrode’s buffer that contained (mM): 131 NaCl, 4 KCl, 1 MgCl$_2$, 10 Heps, and 10 glucose, and was supplemented with 2% bovine serum albumin, and had a pH of 7.4 at 37°C. Cells were initially washed with Ca$^{2+}$-free Tyrode’s buffer to remove remnant enzyme and extracellular Ca$^{2+}$ was added incrementally back to 1.25 mM. Myocytes with obvious sarcolemmal blebs or spontaneous contractions were not used. Only rod-shaped myocytes with clear edges were selected for recording of mechanical properties or intracellular Ca$^{2+}$ transients as previously described.

2.4. Cell shortening/relengthening

Mechanical properties of ventricular myocytes were
were placed in a Warner chamber mounted on the stage of an inverted microscope (Olympus, X-70) and superfused (~1 ml/min at 37°C) with a buffer containing (in mM): 131 NaCl, 4 KCl, 1 CaCl₂, 1 MgCl₂, 10 glucose, 10 Hepes (pH 7.4). The cells were field stimulated with suprathreshold voltage at a frequency of 0.5 Hz and 3 ms duration, using a pair of platinum wires placed on opposite sides of the chamber connected to a FHC stimulator (Brunswick, NE, USA). The myocyte being studied was displayed on the computer monitor using an IonOptix MyoCam camera. The SOFTEDGE software (IonOptix) was used to capture changes in cell length during shortening and relengthening.

2.5. Intracellular fluorescence measurement

A separate cohort of myocytes was loaded with fura-2/AM (0.5 μM) for 10 min and fluorescence measurements were recorded with a dual-excitation fluorescence photon-multiplier system (Ionoptix) as previously described [10]. Myocytes were placed on an Olympus X-70 inverted microscope equipped with a heated (37°C) chamber and imaged through a Fluor×40 oil objective. Cells were exposed to light emitted by a 75-W lamp and passed through either a 360- or a 380-nm filter (bandwidths were ±15 nm), while being stimulated to contract at 0.5 Hz. Fluorescence emissions were detected between 480 and 520 nm by a photomultiplier tube after first illuminating cells at 360 nm for 0.5 s then at 380 nm for the duration of the recording protocol (333 Hz sampling rate). The 360 nm excitation scan was repeated at the end of the protocol and qualitative changes in intracellular Ca²⁺ concentration ([Ca²⁺]) were inferred from the ratio of the fluorescence intensity at two wavelengths.

2.6. Experimental protocol

Myocytes (either fura-2 loaded or non-loaded) were allowed to contract at a stimulation frequency of 0.5 Hz over 10 min to ensure steady state before superfusing with different concentrations of IGF-1 (recombinant human IGF-1, 10⁻¹⁰−10⁻⁶ M) for 5 min. Cells were then washed with normal contractile buffer for 5 min. In some experiments, myocytes were pre-incubated with IGF-1 receptor antagonist H-1356 (20 μg/ml for 4 h), protein kinase inhibitors wortmannin (100 μM) or genistein (50 μM) for 15 min prior to exposure of IGF-1. These agents were also present in the Hepes contractile buffer during respective experiments. The effect of elevated extracellular Ca²⁺ on myocyte shortening was evaluated with an increase of 1 mM Ca²⁺. Data from this laboratory suggested that there is a linear relationship of extracellular Ca²⁺ and shortening when Ca²⁺ is below 4 mM, whereas a saturated response is usually achieved between 3.5 and 4 mM. Myocytes with rundown >10% in peak twitch amplitude over the first 5 min were not studied further.

2.7. Statistical analyses

For each experimental series, data are presented as mean±S.E.M. Statistical significance (P<0.05 or P<0.01) for each variable was estimated by analysis of variance (ANOVA) or t-test, where appropriate (Systat, Evanston, IL, USA).

3. Results

3.1. General features of BB/DR and BB/DP rats

Prolonged spontaneously diabetic state significantly slowed down body weight gain and elevated plasma glucose levels in BB/DP rats compared to the BB/DR littermates. Although the absolute heart weight was less than the BB/DR controls, the heart/body weight ratio was significantly greater in BB/DP rats. Diabetic animals also exhibited hepatomegaly and renal hypertrophy (Table 1).

3.2. Glucose tolerance test

Under continuous Linplant insulin treatment, the 12-h fasting plasma glucose levels in BB/DP rats were still significantly higher than the levels of BB/DR group. Following the intraperitoneal glucose challenge, the plasma glucose levels in BB/DR rats returned to the baseline value after 120 min. However, the post-challenge hyperglycemia was considerably greater in BB/DP rats at 15, 60 and 120 min compared to those of from the non-diabetic rats (Fig. 1). These glucose tolerance tests were conducted at about 11 months of age and have clearly indicated glucose intolerance in these genetically predisposed diabetic rats.

<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>Serum glucose (mM)</th>
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<tbody>
<tr>
<td>BB/DR (8)</td>
<td>496±19</td>
<td>1.72±0.08</td>
<td>3.46±0.12</td>
<td>35.8±1.0</td>
<td>7.0±0.4</td>
<td>5.1±0.8</td>
</tr>
<tr>
<td>BB/DP (7)</td>
<td>348±19**</td>
<td>1.46±0.08*</td>
<td>4.21±0.08**</td>
<td>42.1±1.9**</td>
<td>9.7±0.5**</td>
<td>22.1±1.0**</td>
</tr>
</tbody>
</table>

*Wt., weight; n, number of animals; values represent mean±S.E.M. *P<0.05, **P<0.01 vs. BB/DR group.

Table 1

General features of BB/DR and BB/DP rats
The effect of IGF-1 on cell shortening reached maximal at 5 min of exposure and was partially reversible upon washout. Similar to our earlier report on STZ-induced diabetes [10], IGF-1 failed to exert any response in myocytes isolated from diabetic animals. Lastly, IGF-1 did not affect TPS and TR90 over the concentration range tested (Table 2).

3.4. Effect of IGF-1 on intracellular Ca2+ ([Ca2+]i) transients

To determine whether the differential response of IGF-1 in BB/DR and BB/DP rat cardiac myocytes was due to changes in intracellular Ca2+ concentration, we used fluorescence dye fura-2 to estimate [Ca2+]i in the myocytes from both groups. The time course of the fluorescence signal decay (τ; the duration where 2/3 Ca2+ transient decays from the peak level) was calculated to assess intracellular Ca2+ clearing rate. Myocytes from both groups exhibited similar clearing rates, whereas the BB/DP myocytes displayed significantly lowered baseline FFI (representing resting intracellular Ca2+ levels). Reduced intracellular Ca2+ levels have also been reported in chemically-induced diabetes [17,18]. Interestingly, acute IGF-1 exposure caused a concentration-dependent increase in intracellular Ca2+ (ΔFFI=peak FFI−baseline FFI) in myocytes from both BB/DR and BB/DP animals (Fig. 3). The threshold where IGF-1 exerted a significant increase in Ca2+ transient was between 10−9 and 10−7 M. ΔFFI achieved steady state at or before 5 min and cells recovered partially following washout. Neither resting FFI nor the τ value was affected by IGF-1 (Table 3). The disparate response of IGF-1 on myocyte shortening and intracellular Ca2+ in BB/DP myocytes suggests reduced myofibril responsiveness to intracellular Ca2+ in BB diabetic hearts.

3.5. Effect of IGF-1 on myocyte shortening in the presence of H-1356

To determine the membrane receptor(s) and post-receptor mechanism(s) involved in the IGF-1-induced car-

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Table 2

<table>
<thead>
<tr>
<th>Control</th>
<th>Concentration of IGF-1 (M)</th>
<th>10−10</th>
<th>10−9</th>
<th>10−8</th>
<th>10−7</th>
<th>10−6</th>
</tr>
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<tbody>
<tr>
<td>TPS (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BB/DR</td>
<td></td>
<td>73±3</td>
<td>74±3</td>
<td>81±8</td>
<td>79±5</td>
<td>82±5</td>
</tr>
<tr>
<td>BB/DP</td>
<td></td>
<td>146±6*</td>
<td>139±7*</td>
<td>144±7*</td>
<td>144±7*</td>
<td>155±7*</td>
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<tr>
<td>TR90 (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BB/DR</td>
<td></td>
<td>116±9</td>
<td>128±12</td>
<td>136±11</td>
<td>135±14</td>
<td>146±15</td>
</tr>
<tr>
<td>BB/DP</td>
<td></td>
<td>274±20*</td>
<td>272±25*</td>
<td>268±28*</td>
<td>288±28*</td>
<td>291±28*</td>
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</tbody>
</table>

*Time-to-peak shortening (TPS) and time-to-90% relengthening (TR90). Data represent mean±S.E.M.

*P<0.01 vs. BB/DR group; n=33 cells/group.
Fig. 2. (A) Typical experiments showing the effect of IGF-1 (10^{-7} M) on myocyte shortening in cells isolated from BB/DR (left) and BB/DP (right) hearts; solid and dotted traces: before and 5 min after IGF-1 addition. PS in diabetic cell was normalized to that of non-diabetic myocyte to better illustrate prolongation of shortening and relengthening in BB/DP myocytes. (B) Concentration-dependent response of IGF-1 (10^{-10}–10^{-6} M) on PS in ventricular myocytes from BB/DR or BB/DP animals. Data are presented as percent change from the respective control value. The number of myocytes is given in parentheses. Mean±S.E.M.; *, P<0.05 vs. control, #, P<0.05 vs. BB/DR.

Diabetic contractile response, myocyte shortening in response to IGF-1 was re-examined in the presence of IGF-1 analogue, H-1356 (Bachem Bioscience, King of Prussia, PA, USA). This peptide inhibits the autophosphorylation of the IGF-1 receptor by IGF-1 and therefore decreases its activity [19]. To block the action of IGF-1, H-1356 (20 μg/ml) was pre-incubated with the cardiomyocytes for 4 h and was presented throughout the experimental protocol. Not surprisingly, the IGF-1-induced positive response in cell shortening in BB/DR myocytes was completely blunted (even with slight inhibition) by H-1356 (Fig. 4A). These results indicated the IGF-1-induced cardiac contractile response is likely to be mediated through the IGF-1 receptor specifically.

3.6. Effect of IGF-1 on myocyte shortening in the presence of wortmannin and genistein

To elucidate the mechanism of action involved in IGF-1-induced cardiac contractile response, inhibitors of different pathways were added to cardiac myocytes before incubation with IGF-1. The potential roles of phosphatidylinositol-3 (PI-3) kinase or tyrosine kinase activation in mediating IGF-1-induced cardiac response were studied in myocytes incubated with PI-3 inhibitor wortmannin (100 μM) or tyrosine kinase inhibitor genistein (50 μM). In these experiments the average resting CL of myocytes used was 119±4 and 122±4 μm in the BB/DR and BB/DP groups, respectively. The PS in response to electrical stimulation was 5.4±0.4% (BB/DR) and 6.0±0.5% (BB/DP) (16 cells/group, P>0.05). As shown in Fig. 4B, the IGF-1-induced potentiation in cell shortening in myocytes from BB/DR hearts was greatly attenuated by wortmannin but not genistein. The IGF-1 induced contractile profile in BB/DP hearts was not affected with the presence of kinase antagonists. Neither wortmannin nor genistein alone had any effect on myocyte shortening.

3.7. Effect of insulin, norepinephrine, KCl and increase of extracellular Ca^{2+} on myocyte shortening

For comparison, the contractile responses of insulin (100 nM), norepinephrine (NE, 1 μM) and KCl (30 mM) were examined in myocytes from both BB/DR and BB/DP groups (Fig. 5). Myocytes from BB/DP group exhibited significantly enhanced contractile response to insulin, comparable response to KCl and reduced response to NE,
Fig. 3. Effect of IGF-1 on intracellular Ca\(^{2+}\) transient changes in ventricular myocytes from BB/DR or BB/DP rat hearts. Actual data point in the concentration-dependent response curves were obtained by normalizing intracellular Ca\(^{2+}\) transient changes (ΔFFI) to the respective control value. (A) Typical traces showing the effect of IGF-1 (10\(^{-7}\) M) on intracellular Ca\(^{2+}\) transient before and 5 min after IGF-1 addition in myocytes isolated from BB/DR and BB/DP hearts. (B) Concentration-dependent response of IGF-1 (10\(^{-10}\)–10\(^{-6}\) M) on ΔFFI. Mean±S.E.M., * P<0.05 vs. baseline. Cell number is given in parentheses.

compared to those from BB/DR group. Elevation of extracellular Ca\(^{2+}\) concentration by 1 mM increased the myocyte shortening by 69% in BB/DR myocytes. However, a similar maneuver only increased the myocyte shortening by 10% in myocytes isolated from BB/DP rat hearts (Fig. 5). These data indicated a depressed α-adrenergic action and altered insulin responsiveness as well as myofibril Ca\(^{2+}\) sensitivity in heart from spontaneously diabetic rats, which may underscore the differential IGF-1-induced contractile response between the two groups.

4. Discussion

IGF-1 promotes cardiac growth, improves cardiac contractility, cardiac output, stroke volume, and ejection fraction. It also facilitates glucose metabolism, lowers insulin level, increases insulin sensitivity, and improves the lipid profile, suggesting both a physiological role and a therapeutic potential of the hormone. IGF-1 and its binding proteins have been considered as markers for the presence of certain cardiac abnormalities [12]. Altered responsive-

<table>
<thead>
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<th>Control</th>
<th>Concentration of IGF-1 (M)</th>
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<tbody>
<tr>
<td></td>
<td>10(^{-10})</td>
</tr>
<tr>
<td>Resting FFI</td>
<td></td>
</tr>
<tr>
<td>BB/DR</td>
<td>1.03±0.05</td>
</tr>
<tr>
<td>BB/DP</td>
<td>0.91±0.02*</td>
</tr>
<tr>
<td>τ (ms)</td>
<td></td>
</tr>
<tr>
<td>BB/DR</td>
<td>189±11</td>
</tr>
<tr>
<td>BB/DP</td>
<td>209±8</td>
</tr>
</tbody>
</table>

* Data represent mean±S.E.M.
* P<0.05 vs. BB/DR, n=16 cells/group.
Fig. 4. (A) Effect of IGF-1 analogue H-1356 (20 μg/ml) on IGF-1-induced myocyte contractile response in BB/DR (left panel) or BB/DP (right panel) myocytes. (B) Effect of protein kinase inhibitors wortmannin (100 μM) and genistein (50 μM) on IGF-1-induced myocyte contractile response in BB/DR (left panel) or BB/DP (right panel) myocytes. The number of cells is given in parentheses. Mean ± S.E.M. * P < 0.05 vs. baseline.

One of the important findings of this study is the prolonged duration of myocyte shortening and relengthening, associated with reduced baseline intracellular Ca^{2+} level (FFI) in genetically predisposed diabetes. Prolonged duration of cardiac contraction and relaxation has been documented in chemically-induced diabetes [5,6], and in working heart of genetic diabetes [16]. Several factors have been suggested to contribute to such defects, including depressed activity of sarco(endo)plasmic ATPase (SERCA) [24] and Na–Ca exchange [25], as well as shift of contractile protein myosin isoforms (from fast type V_{i} to slow type V_{s}) [26]. The reduction of resting FFI observed in this study is also consistent with the reports from the chemically-induced diabetes [18]. Although there is still
some controversy regarding the underlying mechanisms, the depressed resting Ca\(^{2+}\) level in diabetes is believed to be associated, at least in part, with the reduced sarcoplasmic Ca\(^{2+}\) stores, transmembrane Ca\(^{2+}\) uptake and Na–H exchange due to intracellular pH change [17,18].

IGF-1 enhances myocardial contractility [10, 20, 27] and is considered as a more important cardiac hormone than insulin. Although the mechanisms of action involved in IGF-1-induced myocardial function have not yet been fully studied, several potential mechanisms may be worthy of consideration including IGF-1-induced IP\(_3\) accumulation, which leads to increases in intracellular Ca\(^{2+}\) concentration [28], and IGF-1-induced increase in intracellular Ca\(^{2+}\) sensitivity, which results in an enhanced contractile responsiveness [29]. In the present study, IGF-1 increased myocyte shortening in myocytes from BB/DR but not BB/DP rats. This disparate pattern was not associated with similar effect in intracellular Ca\(^{2+}\) transients. It is possible that one or more of the following explanations may be responsible for the discrepancy. (1) IGF-1-induced positive responses in BB/DR animals may be related to an increase in intracellular Ca\(^{2+}\) level. This increase in cardiac contraction may be abrogated by a reduced intracellular Ca\(^{2+}\) sensitivity in BB/DP myocytes, which has been reported in diabetes [30]. This was supported also by the observation of a reduced responsiveness to increase of extracellular Ca\(^{2+}\) (Fig. 5). (2) The diabetes-associated alteration(s) of certain IGF-1 receptor-mediated signaling pathways, such as protein kinases, may also contribute to changes in IGF-1-induced cardiac responses, although there is little evidence available at this time.

The positive cardiac contractile effect of IGF-1 was blocked by IGF-1 antagonist H-1356, which itself depresses cardiac contraction. This data suggested that IGF-1-induced cardiac response is likely to be mediated through the IGF-1 receptor, instead of insulin receptor. The direct evidence from insulin showed an augmented responsiveness in spontaneous diabetes, opposite to that exerted by IGF-1 (Fig. 5). The fact that H-1356 inhibited cardiac contraction may suggest a role for endogenous IGF-1 production by cardiac myocytes in determining myocardial contractile response. Circulating IGF-1 may act as an endogenous regulator of myocardial contractility. The rapid onset, the long duration of effect, and the relatively modest magnitude of its action compared with other endogenous substances suggest that if IGF-1 has any acute cardioregulatory role, it may contribute to the modulation of the inotropic responsiveness of the myocardium over a time frame of minutes to hours, sensitizing the myofilaments to rises in [Ca\(^{2+}\)], induced by more potent but short-lived neurohumoral factors.

The fluorescent recording obtained from the current study differed from those we reported recently in chemically-induced diabetes [10], in which IGF-1 failed to elicit any response in intracellular Ca\(^{2+}\) transients in cardiac myocytes from diabetic rats. This apparent discrepancy in cardiac excitation–contraction (E–C) coupling may be largely due to distinct subcellular mechanisms, such as SR function, contractile proteins and certain signal pathways, between the genetically predisposed and the chemically-induced diabetic models. However, little direct evidence is available at this point and further study is warranted to better explore such discrepancy in E–C coupling.

The reduced contractile response of cardiac myocyte to increase of intracellular Ca\(^{2+}\) observed after IGF-1 exposure in BB/DP hearts may reflect potential alteration in the intracellular signaling pathway(s) that can be activated by the IGF-1-receptor complex in diabetes. The IGF-1 signaling cascades are initiated with binding of IGF-1 to its membrane receptors, which are associated with Crk, Shc and insulin substrate-1 (IRS-1). Phosphorylation of Crk, Shc and IRS-1 leads to the activation of Ras, and the sequential activation MAP kinase kinase and MAP kinases. MAP kinases activate a number of transcription factors. Alternatively, tyrosine-phosphorylated IRS-1 binds to p85 regulatory subunit of PI-3 kinase and then activates the catalytic function of the 110-kDa subunit of PI-3 kinase [23, 31]. PI-3 kinase is essential for the transduction of metabolic growth and functional effects of IGF-1 and insulin, including stimulation of glucose transport [32, 33], antilipolysis [33], protein and glycogen synthesis [34], inhibition of apoptosis [35], and more recently, IGF-1 mediated cardiomyocyte contractility [29]. Activation of phospholipase C [36] and rapid elevation of intracellular inositol phosphate levels [28] have been also reported in cardiac cells. Although the linkage of above mentioned signaling cascades to the altered modulation of cardiac contractility of IGF-1 under diabetes is not clear, the present study strongly suggested the involvement of PI-3
kinase in the positive cardiac contractile action elicited by IGF-1. It is possible that PI-3 kinase may mediate, at least in part, the cardiac contractile responses, possibly by interacting with the E–C coupling process. Altered cardiac E–C coupling has been consistently demonstrated in both clinical and experimental diabetes [4–6], and has also been demonstrated in the present study (prolonged TPS, TR 90 and lowered resting Ca2+ level). Whether the PI-3 kinase pathway play a role in the altered E–C coupling, and whether if it plays a role in the regulation of IGF-1-induced cardiac contractile alteration in diabetes, is crucial to our understanding of diabetes-related heart diseases and deserves further investigation.

In summary, the present study demonstrated the presence of resistance to IGF-1-induced cardiac contractile responses in diabetes of genetic origin, similar to that of chemically induced diabetes. This attenuated action may be associated with altered IGF-1 receptor-mediated signaling pathway(s) and/or reduced myofilament Ca2+ responsiveness. Abnormal IGF-1 level or function has been considered to be an important predisposing factor in diabetes-related cardiovascular complications. Future studies should be focused more on the understanding of the cellular mechanisms associated with IGF-1-induced cardiac response and alterations of this response under diabetes.

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