Transgenic overexpression of insulin-like growth factor I prevents streptozotocin-induced cardiac contractile dysfunction and β -adrenergic response in ventricular myocytes

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Abstract

Diabetic cardiomyopathy is characterized by cardiac dysfunction and altered level/function of insulin-like growth factor I (IGF-I). Both endogenous and exogenous IGF-I have been shown to effectively alleviate diabetes-induced cardiac dysfunction and oxidative stress. This study was designed to examine the effect of cardiac overexpression of IGF-I on streptozotocin (STZ)-induced cardiac contractile dysfunction in mouse myocytes. Both IGF-I heterozygous transgenic mice and their wild-type FVB littermates were made diabetic with a single injection of STZ (200 mg/kg, i.p.) and maintained for 2 weeks. The following mechanical indices were evaluated in ventricular myocytes: peak shortening (PS), time-to-PS (TPS), time-to-90% re-lengthening (TR₉₀) and maximal velocity of shortening/relengthening ($\pm$ dL/dt). Intracellular Ca^{2+} was evaluated as resting and peak intracellular Ca^{2+} levels, Ca^{2+} -induced

Ca^{2+} release and intracellular Ca^{2+} decay rate (τ). STZ led to hyperglycemia in FVB and IGF-I mice. STZ treatment prolonged TPS and TR₉₀, reduced Ca^{2+} -induced Ca^{2+} release, increased resting intracellular Ca^{2+} levels and slowed τ associated with normal PS and $\pm$ dL/dt. All of which, except the elevated resting intracellular Ca^{2+} , were prevented by the IGF-I transgene. In addition, myocytes from STZ-treated FVB mice displayed an attenuated contractile response to the β -adrenergic agonist isoproterenol, which was restored by the IGF-I transgene. Contractile response to the α -adrenergic agonist phenylephrine and angiotensin II was not affected by either STZ treatment or IGF-I. These results validate the beneficial role of IGF-I in diabetic cardiomyopathy, possibly due to an improved β -adrenergic response.

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Introduction

Diabetes mellitus is associated with an elevated cardiovascular morbidity and mortality largely due to diabetes-related structural and functional damage in the heart (Fein 1990, Ren *et al.* 1999). Diabetic cardiomyopathy is believed to contribute predominantly to the enhanced likelihood of cardiac death in diabetics independent of macro/microvascular complications (Fein 1990). The most prominent defects of diabetic cardiomyopathy include compromised ventricular functions such as prolonged duration of contraction and relaxation and reduced cardiac compliance (Fein 1990, Ren & Davidoff 1997, Ren & Bode 2000). The etiology of diabetic cardiomyopathy is rather complex and may involve metabolic derangements, depressed autonomic function and abnormalities in certain hormones or proteins that regulate intracellular ion homeostasis, particularly Ca^{2+} (Fein 1990, Rodrigues *et al.* 1998, Norby *et al.* 2002). Nevertheless, either endogenous overexpression or exogenous administration of a key cardiac surviving factor, insulin-like

growth factor I (IGF-I) has exhibited beneficial effects on diabetes-induced cardiac mechanical dysfunction and oxidative damage (Kajstura *et al.* 2001, Norby *et al.* 2002). IGF-I, an insulin analogue and replacement for glucose control in diabetes, promotes cardiac growth and myocardial contraction, improves hemodynamics and energy metabolism, and protects the heart against apoptosis induced by ischemia or oxidative stress (Wang *et al.* 1998, Leri *et al.* 1999, Ren *et al.* 1999, Kajstura *et al.* 2001). The cardiac protective capacity of IGF-I is believed to be mediated through the phosphatidylinositol-3 (PI-3)-kinase-mediated activation of the serine–threonine kinase Akt (Coffer *et al.* 1998, Ren *et al.* 2003). Resistance to IGF-I-induced cardiac response has been shown in chemically induced and genetically predisposed diabetic conditions, or *in vitro* hyperglycemic conditions (Ren *et al.* 1999, Ren 2000, Ren *et al.* 2003). In addition, altered cardiac IGF-I/IGF-I receptor levels have been reported in diabetic patients and experimental animals, suggesting a critical role of IGF-I in diabetic cardiomyopathy (Bornfeldt *et al.* 1992, Bereket *et al.* 1999, Duan *et al.* 2003).

To further test the role of transgenic overexpression of IGF-I on cardiomyocyte function in diabetes, experimental diabetes was induced with a single injection of streptozotocin (STZ) in mice heterozygous for the IGF-I transgene or their wild-type FVB littermates (Reiss *et al.* 1996). State-of-the-art video-based edge-detection and intracellular Ca^{2+} fluorescent imaging techniques were used to examine the mechanical properties in isolated ventricular myocytes. Since our goal was to characterize the impact of IGF-I on the development of diabetic cardiomyopathy, using cardiac specific IGF-I transgenic mice should help to avoid any undesired systemic effects as a result of exogenous IGF-I administration, which may have secondary effects on the hearts.

Materials and Methods

Experimental animals

The experiments described here were approved by the Institutional Animal Care and Use Committees of University of North Dakota (Grand Forks, ND, USA) and University of Wyoming (Laramie, WY, USA). All animal procedures were in accordance with NIH animal care standards.

Eight to ten week-old male IGF-I heterozygous transgenics and their weight-matched wild-type FVB littermates (offspring from male heterozygous IGF-I transgenic mice cross to female FVB mice) were injected with streptozotocin (STZ, 200 mg/kg, i.p.). The pigmentation of fur color (light brown) was used as a marker for heterozygous IGF-I or wild-type FVB mouse identification as described in Reiss *et al.* (1996). Mice were then maintained for 2 weeks with free access to standard lab chow and tap water. Blood glucose levels were measured weekly using a glucose monitor (Accu-ChekII, model 792, Boehringer Mannheim Diagnostics, Indianapolis, IN, USA).

Ventricular myocytes isolation procedures

Hearts were rapidly removed from anesthetized mice and immediately mounted on a temperature-controlled (37 °C) Langendorff perfusion system. After perfusion with modified (Ca^{2+} free) Tyrode solution (pH 7.4, containing (in mM): 135 NaCl, 4 KCl, 1 MgCl_2 , 10 HEPES, 0.33 NaH_2PO_4 , 10 glucose, 10 butanedione monoxime, and gassed with 5% CO_2 -95% O_2) for 2 min, the heart was digested for 10 min with 0.9 mg/ml collagenase D (Boehringer Mannheim Biochemicals) in modified Tyrode solution. The digested heart was then removed from the cannula and the left ventricle was cut into small pieces in the modified Tyrode solution. These pieces were gently agitated and the pellet of cells was resuspended in modified Tyrode solution and allowed to

settle for another 20 min at room temperature during which time extracellular Ca^{2+} was added incrementally back to 1.20 mM. Isolated myocytes were used for experiments within 8 h of isolation. Only rod-shaped myocytes with clear edges were selected for recording of mechanical properties and intracellular Ca^{2+} transients as described in Duan *et al.* (2003).

Cell shortening/relengthening

Mechanical properties of ventricular myocytes were assessed using a SoftEdge MyoCam system (IonOptix Corporation, Milton, MA, USA) (Duan *et al.* 2003). In brief, cells were placed in a Warner chamber mounted on the stage of an inverted microscope (Olympus IX-70, Olympus Optical Corporation, Tokyo, Japan) and superfused (~ 1 ml/min at 25 °C) with a buffer containing (in mM): 131 NaCl, 4 KCl, 1 CaCl_2 , 1 MgCl_2 , 10 glucose, 10 HEPES at pH 7.4. The cells were field stimulated with suprathreshold voltage at a frequency of 0.5 Hz, 3 msec duration, using a pair of platinum wires placed on opposite sides of the chamber connected to a FHC stimulator (FHC Incorporation, Bowdoinham, ME, USA). The myocyte being studied was displayed on the computer monitor using an IonOptix MyoCam camera. SoftEdge software was used to capture changes in cell length during shortening and relengthening.

Intracellular fluorescence measurement

A separate cohort of murine myocytes was loaded with fura-2/AM (0.5 μM) for 10 min and fluorescence measurements were recorded with a dual-excitation fluorescence photomultiplier system (Ionoptix) as described in Duan *et al.* (2003). Myocytes were placed on an Olympus IX-70 inverted microscope and imaged through a Fluor $\times 40$ 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 the cells at 360 nm for 0.5 sec 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 Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) were inferred from the ratio of the fluorescence intensity at two wavelengths.

Statistical analyses

Nine mice were used in each of the four groups. Windows of 10–18, 6–10, and 3–5 cardiac myocytes were evaluated for cell shortening, intracellular Ca^{2+} transient and drug response, respectively, from each mouse. Mechanical results from multiple myocytes were pooled and shown as

Table 1 General features of normal or STZ-induced diabetic FVB and IGF-1 mice

	Mouse group			
	FVB (n=9)	IGF-1 (n=9)	FVB-STZ (n=9)	IGF-STZ (n=9)
Body weight (g) [#]	28.3 ± 1.6	28.7 ± 1.2	23.5 ± 1.2**	26.2 ± 1.2
Heart weight (mg)	178 ± 15	284 ± 33*	191 ± 17	199 ± 20
HW/BW (mg/g)	6.20 ± 0.26	10.71 ± 0.39 [#]	8.36 ± 0.91 [#]	7.73 ± 0.84 [#]
Liver weight (g)	1.418 ± 0.078	1.564 ± 0.103	1.34 ± 0.111	1.404 ± 0.112
LW/BW (mg/g)	51.23 ± 2.14	54.19 ± 1.45	56.69 ± 2.31	53.67 ± 3.14
Kidney weight (g)	0.478 ± 0.035	0.549 ± 0.042	0.476 ± 0.021	0.618 ± 0.060 [#]
KW/BW (mg/g)	17.12 ± 0.77	18.97 ± 0.84	20.52 ± 1.04 [#]	23.77 ± 1.90**
Glucose (mM)	6.08 ± 0.55	5.89 ± 0.34	15.79 ± 1.24**	23.95 ± 2.13**

HW, heart weight; LW, liver weight; KW, kidney weight. Means ± S.E.M., * $P < 0.05$ vs all other groups, ** $P < 0.05$ vs FVB and IGF-1 groups (without STZ treatment), [#] $P < 0.05$ vs FVB group (without STZ treatment), *n*, number of animals.

a single data point in each mouse. For each experimental series, data are presented as means ± S.E.M. Statistical significance ($P < 0.05$) for each variable was estimated by 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 the experimental animals

Short-term (2 weeks) diabetes significantly reduced the body weight gain in FVB but not IGF-I mice (although there was a trend of body weight loss). STZ treatment significantly increased blood glucose levels in both FVB and IGF-I mice. Transgenic overexpression of IGF-I itself significantly enhanced heart weight and heart-to-body weight ratio. STZ treatment failed to affect absolute heart weight but significantly enhanced heart-to-body weight and kidney-to-body weight ratio in both mice groups. The IGF-I transgene did not elicit any further effect on organ-to-body weight ratio in addition to those induced by STZ treatment. The liver weights were not affected by either diabetes or the IGF-I transgene (Table 1).

Cell shortening and relengthening properties of myocytes from FVB and IGF-I mice

As shown in Fig. 1, transgenic overexpression of IGF-I did not significantly affect any of the mechanical parameters tested. There was no significant difference in the resting cell length (CL) of ventricular myocytes among normal or diabetic FVB or IGF-I mice. Peak shortening (PS) amplitude normalized to CL was similar in ventricular myocytes among all 4 groups examined. Myocytes from the diabetic FVB mice demonstrated significantly prolonged time-to-peak shortening (TPS) and time-to-90%-relengthening (TR₉₀) compared with those from all other groups, which is consistent with our previous findings (Duan *et al.* 2003,

Ye *et al.* 2003). Transgenic overexpression of IGF-I in the heart blunted the diabetes-induced prolongation of TPS and TR₉₀. Neither the maximal velocity of shortening (+dL/dt) nor relengthening (−dL/dt) was significantly affected by STZ treatment or IGF-I transgene.

Intracellular Ca²⁺ transient properties in FVB and IGF-I mouse myocytes

We used the membrane permeant form of fura-2/AM to evaluate the properties of intracellular Ca²⁺ transients in cardiomyocytes from normal or diabetic FVB and IGF-I mice. The time course of the fluorescence signal decay was fitted by a single exponential equation, and the time constant (tau) was used as a measure of the cytoplasmic Ca²⁺ clearing rate. The fluorescence measurements revealed that the IGF-I transgene itself significantly reduced the baseline fura-2 fluorescent intensity (representing resting intracellular Ca²⁺ level) without affecting the peak intracellular Ca²⁺ level in response to electrical stimulus, the Ca²⁺-induced Ca²⁺ release (the difference between peak and resting intracellular Ca²⁺ levels) and intracellular Ca²⁺ decay rate. Interestingly, diabetes elevated resting intracellular Ca²⁺, reduced Ca²⁺-induced Ca²⁺ release and slowed the intracellular Ca²⁺ clearing rate without affecting the peak intracellular Ca²⁺ level. All of these diabetes-induced defects in intracellular Ca²⁺ homeostasis, with the exception of resting intracellular Ca²⁺, were effectively prevented by the cardiac overexpression of IGF-I transgene (Fig. 2).

Effect of phenylephrine, isoproterenol and angiotensin II on myocyte PS

Altered function of adrenergic and angiotensin systems has been documented in diabetes-associated heart dysfunction (Roth *et al.* 1995, Kajstura *et al.* 2001, Ye *et al.* 2003). To compare the function of adrenergic and angiotensin systems in myocytes from normal or diabetic FVB and IGF-I

Figure 1 Contractile properties of ventricular myocytes from normal or STZ-induced diabetic FVB and IGF-I transgenic mice. (a) Resting cell length. (b) Peak shortening (PS as % of resting cell length). (c) Time-to-peak shortening (TPS). (d) Time-to-90% relengthening (TR_{90}). (e) Maximal velocity of shortening ($+dL/dt$). (f) Maximal velocity of relengthening ($-dL/dt$). Means $\pm$ S.E.M., $n=9$ mice per group (10–18 myocytes in each mouse), * $P < 0.05$ vs all other groups.

mouse hearts, ventricular myocytes were exposed to the α -adrenergic agonist phenylephrine, the β -adrenergic agonist isoproterenol and angiotensin II for 10 min each. Figure 3 showed that phenylephrine (10 and 100 μM) significantly depressed PS, which was comparable among all groups. The isoproterenol (1 and 10 μM)-induced positive response in PS, seen in three of the groups, was blunted in myocytes from the FVB-STZ diabetic mice. However, this depressed response was restored by the IGF-I transgene. Finally, angiotensin II (10 and 100 nM) exerted similar patterns of depression in PS in myocytes of normal or diabetic FVB and IGF-I groups. The IGF-I transgene itself did not significantly alter the myocyte contractile response to phenylephrine, isoproterenol or angiotensin II.

Discussion

The present study, using cardiac-specific IGF-I over-expression transgenic mice, confirmed our earlier observations regarding the beneficial effects of IGF-I in diabetic cardiac dysfunction (Kajstura *et al.* 2001, Norby *et al.* 2002). In addition, we found that the IGF-I-associated beneficial effects on diabetic heart dysfunction may be related, at least in part, to the improved β -adrenergic responsiveness in diabetes. Our results did not favor the notion that the α -adrenergic or angiotensin II-associated cardiac contractile response plays a major role in diabetes-induced cardiomyocyte dysfunction or the IGF-I-associated cardiac protection against diabetes.

Figure 2 Intracellular Ca^{2+} properties of ventricular myocytes from normal or STZ-induced FVB and IGF-I transgenic mice. (a) Resting intracellular Ca^{2+} levels. (b) Peak intracellular Ca^{2+} levels. (c) Increase in intracellular Ca^{2+} in response to electrical stimuli. (d) Intracellular Ca^{2+} transient decay rate (tau). Means $\pm$ S.E.M., $n=9$ mice per group (6–10 myocytes in each mouse), * $P<0.05$ vs all other groups, ** $P<0.05$ vs FVB and IGF-I groups (without STZ treatment).

Prolonged contraction and relaxation duration are the hallmarks of diabetic cardiomyopathy (Ren & Davidoff 1997, Ren & Bode 2000, Duan *et al.* 2003). Somewhat similar to our earlier observations, the current study demonstrated prolonged duration of TPS and TR_{90} associated with normal PS amplitude and maximal rate of shortening and relengthening ($\pm$ dL/dt) in STZ-induced diabetes (Ren & Davidoff 1997, Ren & Bode 2000, Duan *et al.* 2003). These mechanical dysfunctions are consistent with our current findings of impaired intracellular Ca^{2+} homeostasis shown as reduced intracellular Ca^{2+} clearing rate and the Ca^{2+} -induced intracellular Ca^{2+} release in STZ-induced diabetic mouse ventricular myocytes. It is worth pointing out that the mechanical as well as intracellular Ca^{2+} defects observed in STZ diabetic mice are similar to those found in diabetic rat models (Ren & Davidoff 1997, Ren & Bode 2000). Several mechanisms have been postulated for these mechanical and intracellular Ca^{2+} defects. It has been reported that the depressed rate of shortening may be associated with diabetes-induced shifts in contractile protein isoforms, such as the shift of myosin heavy chain isoform from the fast type (V_1) to the slow type (V_3) (Dillmann 1989). Diabetes has also been demonstrated to significantly reduce the myofilament Ca^{2+} sensitivity (Hofmann *et al.* 1995, Ren & Bode 2000).

Finally, the slowed intracellular Ca^{2+} decay and prolonged duration of relaxation may simply be a consequence of impaired sarco(endo)plasmic reticulum Ca^{2+} -ATPase (SERCA) and $\text{Na}^+/\text{Ca}^{2+}$ exchanger function (Dillmann 1989, Chattou *et al.* 1999). Although the mechanism behind the higher peak Ca^{2+} response in the IGF-STZ mouse group (Fig. 2b) is not known, a synergistic effect between the IGF-I transgene and short-term STZ treatment may be speculated. Short-term STZ treatment (2 weeks) may transiently upregulate certain intracellular Ca^{2+} regulatory proteins through a compensatory mechanism to reconcile the detrimental cardiac effect of diabetes (Depre *et al.* 2000). While the compensatory mechanism itself may not be sufficient for any effect on intracellular Ca^{2+} handling, it may certainly be synergized with IGF-I, which is known to promote cardiac intracellular Ca^{2+} homeostasis (Ren *et al.* 1999), to significantly enhance the peak Ca^{2+} response in myocytes from IGF-STZ mice.

Our present study revealed that cardiac transgenic overexpression of IGF-I may prevent the diabetes-induced cardiac mechanical dysfunction at the cellular level and protect the β -adrenergic response blunted by STZ diabetic treatment. IGF-I is known to facilitate glucose metabolism, lower insulin levels, improve insulin

Figure 3 Concentration-dependent effects of (a) phenylephrine (10 and 100 μ M), (b) isoproterenol (1 and 10 μ M) and (c) angiotensin II (10–100 nM) on peak cell shortening (PS) in myocytes from normal or STZ-induced FVB and IGF-I transgenic mice. Actual data points were obtained by normalizing PS amplitude to PS value of the respective control. Means $\pm$ S.E.M., $n=9$ mice per group (3–5 myocytes in each mouse), * $P<0.05$ vs baseline, # $P<0.05$ vs all other groups.

sensitivity and lipid profile (Ren *et al.* 1999, Samarel 2002), suggesting both a physiological role and therapeutic potential. 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-I has an acute cardioregulatory role, it may contribute to the modulation of the inotropic responsiveness of the myocardium over a time frame of minutes to hours (Ren *et al.* 1999, Samarel 2002). In addition to its beneficial effect on glucose transport and lipid metabolism, other scenarios have been speculated for IGF-I-induced cardiac protection against stress such as diabetes. IGF-I has been demonstrated to directly regulate membrane ionic channels responsible for cardiac excitation–contraction coupling (Guo *et al.* 1997, Solem & Thomas 1998). Recent evidence suggested that IGF-I-induced cardiac protection against diabetes may involve attenuated p53 function, angiotensin II production and angiotensin receptor activation (Kajstura *et al.* 2001). Our recent study also suggested that IGF-I may rescue the diabetes-induced reduction in SERCA protein abundance (Norby *et al.* 2002). Data from this study suggested that the STZ treatment failed to elicit any further cardiac hypertrophic response in IGF-I transgenic mice. The higher cardiac mass and heart-to-body weight ratio in the IGF-I transgenic mice is consistent with our earlier reports and is likely to be due to a hyperplastic response of myocytes to this growth factor (Reiss *et al.* 1996, Welch *et al.* 2002). The fact that STZ treatment failed to produce any further cardiac hypertrophic response indicates that ventricular remodeling in response to IGF-I may be capable of offsetting the detrimental effect of STZ on cardiac structure and thus offer protection. Although it can be argued that the STZ treatment and IGF-I transgene may ‘mask’ each other in the cardiac hypertrophic response, the fact that STZ and IGF-I induce cardiac hypertrophy through completely different mechanisms makes this ‘masking’ hypothesis rather unlikely. STZ is believed to cause cardiac hypertrophy largely through a genomic adaptive or maladaptive process (Depre *et al.* 2000).

IGF-I has been demonstrated to facilitate cardiac DNA and protein synthesis, reduce protein degradation, and participate in early neonatal cardiomyocyte proliferation and maturation (see Ren *et al.* 1999 for review). IGF-I possesses multiple beneficial effects on cardiac structure and function such as normalization of heart mass, hemodynamics and apoptosis. IGF-I expression acts as a proliferative stimulus to enhance myocyte number through cellular replication (Reiss *et al.* 1996, Welch *et al.* 2002). In transgenic mice overexpressing IGF-I in myocardium, total heart weight is increased by 50% (consistent with our current study) and the myocyte number is increased by 20–50% (Reiss *et al.* 1996). On the contrary, IGF-I deficiency may lead to cardiac atrophy and impaired cardiac function (Isgaard *et al.* 1999, Ren & Brown-Borg 2002). These beneficial effects of IGF-I suggest the crucial

role of IGF-I in cardiac morphology and ventricular function.

Perhaps the most interesting result from our current study is the protected β -adrenergic responsiveness in diabetic myocytes with concurrent IGF-I overexpression, suggesting that protection of β -adrenergic responsiveness may play a role in IGF-I-induced cardiac protection. Our data is consistent with previous findings that isoproterenol increases the contraction amplitude and the velocities of contraction and relaxation in ventricular myocytes (Lefroy *et al.* 1996). Inconsistent observations have been documented regarding the impact of diabetes on the β -adrenergic system. Although β -adrenergic receptor number may not be depressed by diabetes, alteration in certain post-receptor mechanisms such as G-protein and adenylate cyclase are speculated to be responsible for attenuated cardiac β -adrenergic responsiveness in diabetes (Roth *et al.* 1995). The mechanism by which IGF-I restores the depressed β -adrenergic response in diabetes is largely unknown at this time. However, it has been shown that IGF-I directly stimulates phosphorylation of the β -adrenergic receptor *in vivo* on sites distinct from those phosphorylated in response to insulin (Karoo & Malbon 1996).

Our study did not favor any role for angiotensin II and α -adrenergic systems in either diabetes-induced cardiomyocyte dysfunction or the IGF-I-mediated improvement of cardiac mechanical function. Angiotensin II and phenylephrine are receptor agonists with similar signaling mechanisms via acceleration of cardiac phosphoinositide hydrolysis. The regulation of myocardial contractility by these receptor agonists has exhibited a wide range of species-dependent variation. While angiotensin II fails to affect contraction in rat, guinea pigs, or human atrial/ventricular myocytes (Lefroy *et al.* 1996), it produces a sustained negative response in adult mice (Sekine *et al.* 1999). Both angiotensin II and phenylephrine have been shown to inhibit cell shortening but not intracellular Ca^{2+} transients through a protein kinase C-dependent pathway in mouse cardiomyocytes (Sakurai *et al.* 2002). Treatment of mouse papillary muscles with phenylephrine resulted in a sustained reduction in cardiac contractility, whereas a positive inotropic effect occurred in rat papillary muscles (Nishimaru *et al.* 2001, Montgomery *et al.* 2002).

Experimental limitations

Although our study provided evidence that the β -adrenergic response is likely to be protected by the IGF-I transgene under diabetes, we were unable to measure the circulating catecholamine levels and the cardiac β -adrenergic receptor binding properties in diabetic mice carrying IGF-I transgene. Further study is warranted to provide more precise information regarding the role of IGF-I on the adrenergic system in diabetes.

In summary, our findings revealed impaired cardiac contractile function representative of diabetic cardiomyopathy in STZ-induced diabetic mouse hearts, an effect which can be ablated with cardiac overexpression of IGF-I. Our data suggest the improved β -adrenergic response may play a role in the improved mechanical function elicited by IGF-I. Given what we know about the ability of IGF-I to promote cell survival and cardiac performance (Ren *et al.* 1999, Samarel 2002), the in-depth mechanism of action and clinical value of employing IGF-I in the prevention and treatment of diabetic cardiomyopathy warrants further investigation.

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