# Altered inotropic response to IGF-I in diabetic rat heart: influence of intracellular $Ca^{2+}$ and NO

JUN REN, MARY F. WALSH, MARWAN HAMATY,
JAMES R. SOWERS, AND RICARDO A. BROWN
*Departments of Physiology and Internal Medicine, Wayne State University School of Medicine, and John D. Dingell Veterans Affairs Medical Center, Detroit, Michigan 48201*

**Ren, Jun, Mary F. Walsh, Marwan Hamaty, James R. Sowers, and Ricardo A. Brown.** Altered inotropic response to IGF-I in diabetic rat heart: influence of intracellular $Ca^{2+}$ and NO. *Am. J. Physiol.* 275 (*Heart Circ. Physiol.* 44): H823–H830, 1998.—Normally, insulin-like growth factor I (IGF-I) exerts positive effects on cardiac growth and myocardial contractility, but resistance to its action has been reported in diabetes. This study was designed to determine whether IGF-I-induced myocardial contractile action is altered in diabetes as a result of an intrinsic alteration of contractile properties at the cellular level. Contractile responses to IGF-I were examined in left ventricular papillary muscles and ventricular myocytes from normal and short-term (5–7 days) streptozotocin-induced diabetic rats. Mechanical properties of muscles and myocytes were evaluated using a force transducer and an edge detector, respectively. Preparations were electrically stimulated at 0.5 Hz, and contractile properties analyzed include peak tension development (PTD) or peak twitch amplitude (PTA), time to peak contraction/shortening, and time to 90% relaxation/relengthening. Intracellular $Ca^{2+}$ transients were measured as fura 2 fluorescence intensity changes. IGF-I (1–500 ng/ml) caused a dose-dependent increase in PTD and PTA in preparations from normal but not diabetic animals. IGF-I did not alter time to peak contraction/shortening or time to 90% relaxation/relengthening. Pretreatment with the NO synthase inhibitor $N^{\omega}$-nitro-L-arginine methyl ester (100 μM) attenuated IGF-I-induced increases in PTD in normal myocardium but unmasked a positive inotropic action in diabetic animals. Pretreatment with $N^{\omega}$-nitro-L-arginine methyl ester blocked IGF-I-induced increases in PTA in single myocytes. Consistent with its inotropic actions on muscles and myocytes, IGF-I induced a dose-dependent increase in $Ca^{2+}$ transients in normal but not diabetic myocytes. These results suggest that the IGF-I-induced inotropic response is depressed in diabetes because of an intrinsic alteration at the myocyte level. Mechanisms underlying this alteration in IGF-I-induced myocardial response may be related to changes in intracellular $Ca^{2+}$ and/or NO production in diabetes.

insulin-like growth factor I; nitric oxide; papillary muscle; ventricular myocyte; calcium transient

CARDIOVASCULAR MORTALITY is increased among patients with type I and type II diabetes (30), and the incidence of heart failure after myocardial infarction is significantly greater among diabetic than among nondiabetic patients (17). Diabetic cardiomyopathy is a common feature of long-term diabetes and occurs independently of coronary artery disease and/or hypertension (9). Clinically, this diabetes-induced myopathic state is associated mainly with impaired diastolic function (25). In experimental diabetes the mechanical properties of myocardium and cardiomyocytes in vitro are significantly altered, characterized by prolongation of contraction and relaxation as well as a marked slowing in relaxation velocity (3, 4, 10, 23). These diabetes-induced alterations have been attributed to biochemical and ultrastructural anomalies, in particular, impaired intracellular $Ca^{2+}$ homeostasis involving reduced sarcoplasmic $Ca^{2+}$-ATPase activity (19).

Insulin-like growth factor I (IGF-I), a peptide hormone structurally and functionally similar to insulin, is synthesized by various cell types, including cardiomyocytes (1, 22), where it may act as an autocrine/paracrine factor exerting inotropic and growth effects (6). IGF-I is known to stimulate cardiac protein synthesis (12), increase inositol 1,4,5-trisphosphate levels (13), and enhance cell shortening and intracellular $Ca^{2+}$ transients (11, 34), but the precise mechanism of its inotropism has not been elaborated.

Because of the diverse effects of IGF-I, there has been emerging interest in the therapeutic use of this hormone in a variety of disease states, including diabetes (32). We recently demonstrated that short-term diabetes leads to cardiac mechanical dysfunction at the cellular level, such as prolonged shortening and relengthening duration and slowed intracellular $Ca^{2+}$ extrusion, in a manner similar to long-term diabetes at the cellular and tissue levels (4, 23). Because short-term diabetes does not create the ultrastructural changes seen in the sustained diabetic state, it provides a useful model to study early-stage diabetes-induced alterations in contraction, relaxation duration, and intracellular $Ca^{2+}$ handling. Therefore, we characterized the inotropic action of IGF-I in this insulinopenic/hyperglycemic model. We also investigated the potential role of intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) and myocardial NO metabolism in modulating IGF-I-induced inotropic responses in normal and diabetic hearts. We have used isolated left ventricular papillary muscles and ventricular myocytes from short-term diabetic and age-matched normal rats.

## MATERIALS AND METHODS

### *Animals*

The experimental procedures outlined in this study were approved by the Wayne State University Animal Investigation Committee. Briefly, diabetes was induced in male Sprague-Dawley rats (200–225 g; Charles River, Wilmington, MA) with a single injection of streptozotocin (65 mg/kg ip; Sigma Chemical, St. Louis, MO), as previously described

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(2–4). After 5–7 days of untreated diabetes, animals were euthanized under ketamine-xylazine sedation (3:1, 1.32 mg/kg ip). A group of sham-treated age-matched euglycemic Sprague-Dawley rats was selected in parallel with the streptozotocin-treated rats. Hearts were rapidly removed and used for experiments as described below. 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 analyzer (Yellow Springs Instruments, Yellow Springs, OH).

### *Tension Measurement of Papillary Muscle*

Tension development of papillary muscles was measured with a force transducer (model F-30, Hugo Sachs, March-Hugstetten, Germany), as described previously (2–4). After euthanasia, hearts were rapidly excised and immersed in oxygenated (95% $O_2$-5% $CO_2$) Tyrode solution (in mM: 5.4 KCl, 136.9 NaCl, 11.9 $NaHCO_3$, 0.50 $MgCl_2$, 2.70 $CaCl_2$, 0.45 $NaH_2PO_4$, 5.6 glucose, pH 7.4) at 37°C. Left ventricular papillary muscles were dissected and mounted vertically in temperature-controlled organ baths (50 ml) containing Tyrode solution of the same ionic composition. The solution was continuously gassed with 95% $O_2$-5% $CO_2$ through fine pores of sintered glass bubblers. Preparations were allowed to equilibrate in Tyrode solution for 60 min while being electrically driven by a stimulator (model S-88, Grass Instrument, Quincy, MA) at a frequency of 0.5 Hz for establishment of baseline isometric peak tension development (PTD). Square-wave pulses of 4- to 8-ms duration and 50% suprathreshold were delivered through a pair of platinum electrodes in close contact with both ends of the muscle. Isometric tension was recorded at ~90% of maximal length. Signals were amplified, differentiated, and displayed on a chart recorder (model 79, Grass Instrument). The output of the chart recorder was coupled to the input stage of an analog-digital board and later analyzed using a software program (Dasy Lab 3, Biotech Products, Greenwood, IN). PTD was normalized to respective control values and presented as percent increase of isometric tension development to minimize any intermuscle variance. Because the inotropic response to IGF-I was maximal within 3 min of exposure and remained steady for >40 min, all the measurements were taken after a 5-min exposure to the peptide.

### *Cell Isolation Procedures*

Single ventricular myocytes were enzymatically isolated from the hearts using the method described previously (23). 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 $KH_2PO_4$, 25 $NaHCO_3$, 10 HEPES, and 11.1 glucose, 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, then perfused for 20 min with $Ca^{2+}$-free KHB containing 176 U/ml collagenase (Worthington Biochemical, Freehold, NJ) and 0.1 mg/ml hyaluronidase (Sigma Chemical). 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 Chemical) and 0.02 mg/ml deoxyribonuclease (Worthington Biochemical) 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 buffer containing (in mM) 131 NaCl, 4 KCl, 1 $MgCl_2$, 10 HEPES, and 10 glucose, supplemented with 2% BSA, pH 7.4 at 37°C. Cells were initially washed with $Ca^{2+}$-free Tyrode buffer to remove remnant enzyme, and extracellular $Ca^{2+}$ was added incrementally back to 1.25 mM.

Isolated myocytes were then plated on glass coverslips precoated with laminin (10 μg/ml; Collaborative Biochemical Products, Bedford, MA) and maintained for 12–24 h in a serum-free medium consisting of medium 199 (Sigma Chemical) with Earle's salts containing 25 mM HEPES and $NaHCO_3$ supplemented with BSA (2 mg/ml), L-carnitine (2 mM), creatine (5 mM), taurine (5 mM), glucose (5 mM), insulin (0.1 μM), *d*-triiodothyronine (0.1 nM), penicillin (100 U/ml), streptomycin (100 mg/ml), and gentamicin (100 mg/ml). 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 (23).

### *Cell Shortening/Relengthening*

Mechanical properties of cultured ventricular myocytes were assessed using a video-based edge-detection system (Crescent Electronics, Sandy, UT), as described previously (23). Briefly, coverslips with cells attached were placed in a chamber mounted on the stage of an inverted microscope (Nikon Diaphot) and superfused (~2 ml/min at 30°C) with a buffer containing (in mM) 131 NaCl, 4 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 HEPES, pH 7.4. The cells were field stimulated with suprathreshold voltage and at a frequency of 0.5 Hz, 3-ms duration, using a pair of platinum wires placed on opposite sides of the chamber connected to a Grass S88 stimulator. The polarity of stimulatory electrodes was reversed frequently to avoid possible buildup of the electrolyte by-products. A video-imaging edge detector (IonOptix, Milton, MA) was used to capture and convert changes in cell length during shortening and relengthening to an analog voltage signal. This voltage signal was subsequently analyzed with pClamp software. The myocyte being studied was displayed on a Sony monitor using a Pulnix camera, which rapidly scans the image area every 8.3 ms, such that the amplitude and velocity of shortening/relengthening are recorded with good fidelity.

### *Intracellular Fluorescence Measurement*

A separate cohort of myocytes was loaded with fura 2-AM (1 μM) for 15 min at 30°C, and fluorescence measurements were recorded with a dual-excitation fluorescence photomultiplier system (IonOptix), as previously described (23). Myocytes were placed on a Nikon Diaphot inverted microscope equipped with a heated (30°C) and light-tight chamber and imaged through a Nikon Fluor ×40 oil objective. Cells were exposed to light emitted by a 75-W lamp and passed through a 360- or 380-nm filter (bandwidths ~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 cells were illuminated 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 $[Ca^{2+}]_i$ were inferred from the ratio of the fluorescence intensity at two wavelengths.

### *Experimental Protocol*

*Papillary muscle tension development.* After the equilibration period the muscles were exposed to IGF-I (1–500 ng/ml, 1 ng/ml ≅ 0.17 nM; Genentech) for 5 min. This dose range has

Table 1. *General features of normal and diabetic rats*

| Group | *n* | Body Wt, g | Heart Wt, g | Heart Wt/Body Wt, mg/g | Liver Wt/Body Wt, mg/g | Kidney Wt/Body Wt, mg/g |
|---|---|---|---|---|---|---|
| Normal | 18 | 370 ± 15 | 1.10 ± 0.05 | 3.05 ± 0.08 | 38.8 ± 1.3 | 8.0 ± 0.3 |
| Diabetic | 17 | 269 ± 17* | 0.92 ± 0.06* | 3.03 ± 0.12 | 47.0 ± 1.6* | 12.5 ± 0.5* |

Values are means ± SE; *n*, number of animals. *$P < 0.05$ vs. normal.

previously been used for contractility studies (11, 35). Agents were added cumulatively to compose a dose-response curve. Recovery was continuously monitored after removal of the drug from the organ baths. In some studies, $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME, 100 μM; Sigma Chemical) was incubated with the muscles for 15 min before IGF-I addition. The following parameters were measured: PTD, time to peak tension (TPT), time to 90% relaxation ($RT_{90}$), and maximum velocity of tension development and decay (+VT and −VT, respectively). Papillary muscles were under continuous electrical stimulation at 0.5 Hz throughout the experiments. Muscles with PTD <1 g or any rundown were not selected for study.

*Myocyte mechanics.* Myocytes (fura 2 loaded or nonloaded) were allowed to contract at a stimulation frequency of 0.5 Hz over 10 min to ensure steady state before superfusion with IGF-I for 5 min and continuously thereafter. Cells were then washed with normal contractile buffer for 5 min. In some experiments, myocytes were perfused with L-NAME-containing buffer before exposure of IGF-I. Myocytes with rundown >10% in peak twitch amplitude (PTA) over the first 5 min were not studied further (23).

### *Data Analysis*

For each experimental series, values are means ± SE. Statistical significance ($P < 0.05$) for each variable was estimated by ANOVA or *t*-test, where appropriate (SYSTAT, Evanston, IL).

## RESULTS

### *Papillary Muscle Tension Development*

*General features of normal and diabetic animals.* Serum glucose levels were elevated by ~1.9-fold ($P < 0.05$) after short-term diabetes (5–7 days). Table 1 shows the effects of experimental diabetes on body, heart, liver, and kidney weights. As previously observed (23), diabetic rats exhibited significantly lower body weights than age-matched normal animals. Although absolute heart weight was smaller in the diabetic group, it was not different from the normal group when assessed as percentage of body weight. Diabetic animals also showed greater proportional liver and kidney weights.

*Inotropic effects of IGF-I on papillary muscle isometric tension development.* There was no significant difference in baseline papillary muscle PTD between the normal and diabetic groups (1.40 ± 0.13 and 1.48 ± 0.08 g, respectively). Even after a short period of experimental diabetes (5–7 days), the myocardium from diabetic animals exhibited a prolonged baseline contraction and relaxation duration compared with normal myocardium, as observed previously at the tissue (2–4) and cellular level (23). However, there was no effect of short-term diabetes on +VT and −VT (Table 2). Representative traces in Fig. 1*A* show that IGF-I (500 ng/ml) significantly increased PTD in myocardium from normal but not diabetic animals. IGF-I exerted a concentration-dependent positive inotropic effect on myocardium from normal but not diabetic rats (Fig. 1*B*). At 1, 10, 100, and 500 ng/ml, IGF-I increased PTD by 1.5 ± 0.8, 5.2 ± 1.3, 7.9 ± 1.8, and 8.0 ± 2.3%, respectively, in papillary muscles from normal rats. The threshold for the inotropic effect of IGF-I in normal myocardium was between 10 and 100 ng/ml. Higher concentration of IGF-I (to 1,000 ng/ml) did not induce further increase of PTD (data not shown). By contrast, IGF-I did not modify PTD in papillary muscles from diabetic animals.

*Effects of IGF-I on TPT, $RT_{90}$, +VT, and −VT.* As mentioned above, the baseline durations of contraction and relaxation were significantly longer in diabetic myocardium (Fig. 1*A*, Table 2). However, IGF-I did not affect the duration of contraction (TPT) and relaxation ($RT_{90}$) or +VT and −VT in normal and diabetic myocardium (Table 2).

*Effect of IGF-I on myocardial contraction in the presence of L-NAME.* To explore possible mechanism(s) of action of IGF-I, the effect of the hormone was reexamined in the presence of the NO synthase (NOS) inhibitor L-NAME (100 μM). L-NAME alone did not affect PTD at the concentration used over a duration of >30 min (0.4 ± 1.4 and −0.1 ± 1.2% in normal and diabetic myocardium, respectively). As shown in Fig. 2*A*, the positive inotropic responsiveness to IGF-I ob-

Table 2. *Influence of IGF-I on baseline contraction and relaxation duration and velocity of tension development or decline in normal and diabetic myocardium*

| | TPT, ms | $RT_{90}$, ms | +VT, g/s | −VT, g/s |
|---|---|---|---|---|
| | | *Normal* | | |
| Basal | 84.5 ± 2.6 | 139.4 ± 4.1 | 22.0 ± 1.7 | −14.6 ± 1.2 |
| IGF-I | | | | |
| 1 ng/ml | 84.6 ± 1.7 | 137.4 ± 3.8 | 22.2 ± 1.8 | −14.6 ± 1.2 |
| 10 ng/ml | 84.6 ± 1.7 | 136.8 ± 3.9 | 23.1 ± 1.8 | −15.3 ± 1.2 |
| 100 ng/ml | 84.5 ± 1.9 | 140.2 ± 3.2 | 23.5 ± 2.0 | −15.3 ± 1.3 |
| 500 ng/ml | 86.0 ± 2.0 | 137.7 ± 3.2 | 23.5 ± 1.9 | −15.3 ± 1.3 |
| | | *Diabetic* | | |
| Basal | 95.8 ± 2.1* | 161.9 ± 5.3* | 21.4 ± 1.1 | −14.0 ± 0.8 |
| IGF-I | | | | |
| 1 ng/ml | 95.2 ± 2.1 | 160.8 ± 5.1 | 21.5 ± 1.1 | −14.1 ± 0.7 |
| 10 ng/ml | 95.3 ± 1.7 | 158.9 ± 5.1 | 21.7 ± 1.1 | −14.1 ± 0.7 |
| 100 ng/ml | 95.7 ± 1.9 | 158.6 ± 5.0 | 21.9 ± 1.1 | −14.2 ± 0.7 |
| 500 ng/ml | 94.6 ± 1.9 | 155.9 ± 4.4 | 22.1 ± 1.2 | −14.4 ± 0.8 |

Values are means ± SE of 16–20 muscles/group. TPT, time to peak contraction; $RT_{90}$, time to 90% relaxation; +VT, maximal velocity of tension development; −VT, maximal velocity of tension decay. *$P <$ 0.05 vs. respective normal.

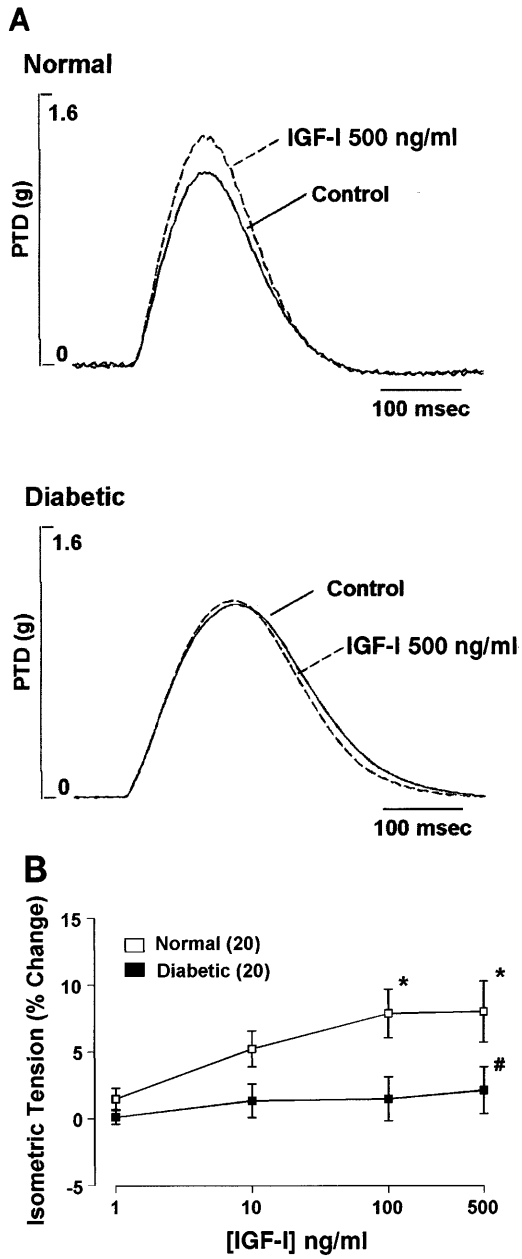


Fig. 1. *A*: typical experiments showing effect of insulin-like growth factor I (IGF-I, 500 ng/ml) on myocardial contraction in papillary muscles isolated from a normal and a diabetic heart. Solid and dashed traces, before and 5 min after IGF-I addition. Peak tension development (PTD) in diabetic myocardium was normalized to that of normal myocardium to better illustrate prolongation of contraction and relaxation duration in diabetes. *B*: inotropic response to IGF-I (1–500 ng/ml) on PTD in papillary muscles from normal and diabetic animals. [IGF-I], IGF-I concentration. Values (means ± SE) are presented as percent change from respective control value, with number of muscles in parentheses. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. normal.

served in myocardium from normal rats was abolished by L-NAME. Interestingly, pretreatment with L-NAME unmasked a positive inotropic response to IGF-I in myocardium from diabetic animals (Fig. 2*B*), suggesting that diabetic myocardium may already manifest substantial tonic NO production.

*Ventricular Myocyte Contraction*

*Effect of IGF-I on myocyte shortening (PTA).* The average resting cell length (CL) of ventricular myocytes used in this study was 129 ± 5 and 108 ± 2 μm in normal and diabetic rats (80 cells/group), respectively. The PTA (normalized to CL) in response to electrical stimulation in myocytes from normal and diabetic hearts was 7.5 ± 0.6 and 8.1 ± 0.6%, respectively. Acute IGF-I exposure did not affect resting myocyte CL over the range of concentrations tested (data not shown). A representative trace depicting the typical effect of IGF-I (100 ng/ml) on cell shortening in myocytes isolated from normal hearts is shown in Fig. 3*B*. At the end of a 5-min exposure to this concentration of IGF-I, PTA was increased by 26.3% with little apparent effect on duration of shortening or relengthening. IGF-I (1–500 ng/ml) caused a concentration-dependent increase in PTA, the threshold of which was between 10 and 100 ng/ml (Fig. 3). The effect of IGF-I on cell shortening was maximal at 5 min of exposure, and cells recovered almost completely on removal of the drug from the superfusate. In line with data from isolated papillary muscle, IGF-I failed to exert a positive response, showing a slight decrease in shortening in

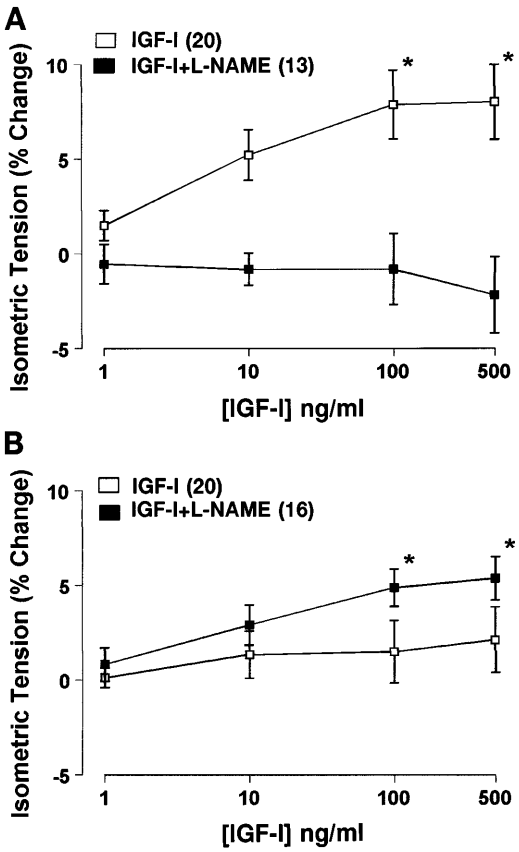


Fig. 2. Effect of $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME) on IGF-I-induced inotropic response in papillary muscles from normal (*A*) and diabetic (*B*) animals. Papillary muscles were pretreated with L-NAME (100 μM) for 15 min before application of IGF-I (1–500 ng/ml). Values (means ± SE) are presented as percent change from respective control value, with number of muscles in parentheses. $^{*}P < 0.05$ vs. control.

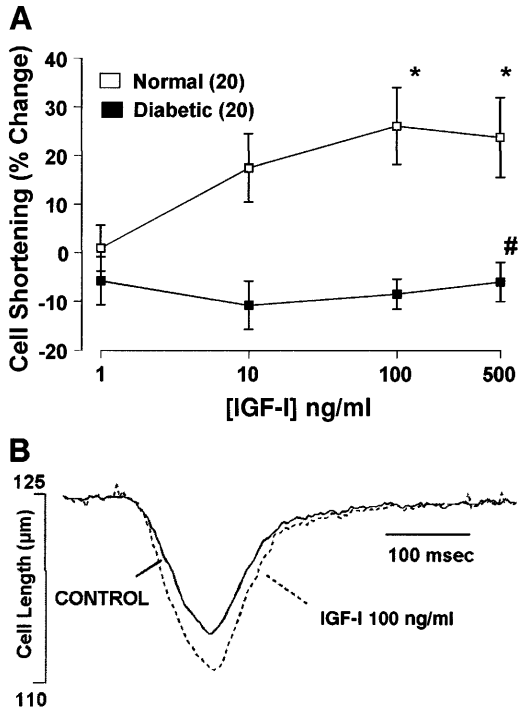


Fig. 3. *A*: concentration-dependent responses to IGF-I (1–500 ng/ml) on cell shortening in normal and diabetic myocytes. *B*: typical experiment showing effect of IGF-I (100 ng/ml) on cell shortening in a myocyte isolated from a normal heart. Solid and dashed traces, before and 5 min after IGF-1 addition. Values (means ± SE) are presented as percent change from respective control value, with number of muscles in parentheses. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. normal.

myocytes isolated from diabetic hearts, indicating that the IGF-I contractile response is markedly attenuated in diabetes.

For comparison, the effect of norepinephrine (1 μM) and KCl (30 mM) on myocyte shortening was also examined. Both agonists significantly increased shortening to a much greater extent (50–84%) in myocytes from normal and diabetic hearts (Fig. 4). Finally, as demonstrated in isolated papillary muscles, the baseline durations of shortening and relengthening were

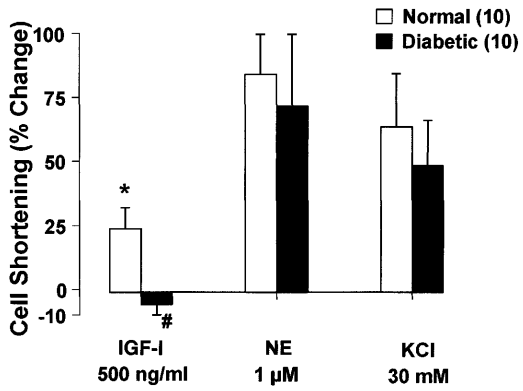


Fig. 4. Effects of norepinephrine (NE, 1 μM) and KCl (30 mM) on cell shortening in normal and diabetic myocytes. Values (means ± SE) are presented as percent change from respective control value, with number of myocytes in parentheses. Inotropic response to IGF-I (500 ng/ml) is also shown for comparison. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. normal.

Table 3. *Effect of IGF-I on duration of myocyte shortening and relengthening in cells from normal and diabetic hearts*

| | Control | IGF-I 1 ng/ml | IGF-I 10 ng/ml | IGF-I 100 ng/ml | IGF-I 500 ng/ml |
|---|---|---|---|---|---|
| TPS, ms | | | | | |
| Normal | 117 ± 3 | 113 ± 6 | 119 ± 8 | 124 ± 8 | 109 ± 4 |
| Diabetic | 131 ± 2* | 135 ± 3 | 126 ± 5 | 133 ± 4 | 124 ± 4 |
| $TR_{90}$, ms | | | | | |
| Normal | 143 ± 5 | 142 ± 12 | 128 ± 9 | 127 ± 9 | 147 ± 8 |
| Diabetic | 160 ± 4* | 164 ± 8 | 164 ± 6 | 168 ± 8 | 150 ± 7 |

Values are means ± SE of 20 cells/group. TPS, time to peak shortening; $TR_{90}$, time to 90% relengthening. $^{*}P < 0.05$ vs. normal.

significantly longer in diabetic myocytes. Consistent with the results in papillary muscles, IGF-I did not modify the duration or velocity of shortening or relengthening (Table 3). This underscores the differential actions of IGF-I compared with other contractile agonists.

*Effect of IGF-I on myocyte shortening in the presence of* L-*NAME*. The effect of IGF-I on myocyte shortening was reexamined in the presence of L-NAME (100 μM). In these experiments the average resting CL of myocytes was 107 ± 4 and 118 ± 6 μm in the normal and

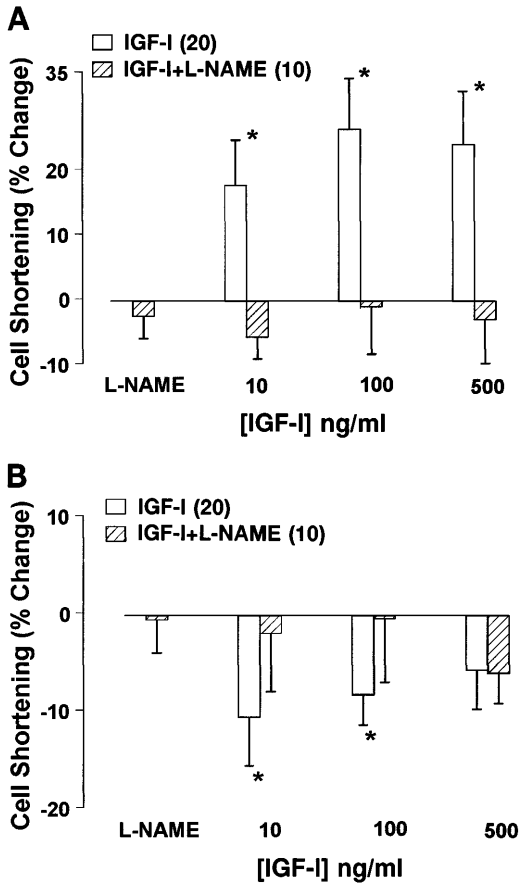


Fig. 5. Effects of L-NAME on IGF-I-induced response on cell shortening in normal (*A*) and diabetic (*B*) myocytes. Myocytes were pretreated with L-NAME (100 μM) for 15 min before application of IGF-I (10–500 ng/ml). Values (means ± SE) are presented as percent change from respective control value, with number of myocytes in parentheses. $^{*}P < 0.05$ vs. control.

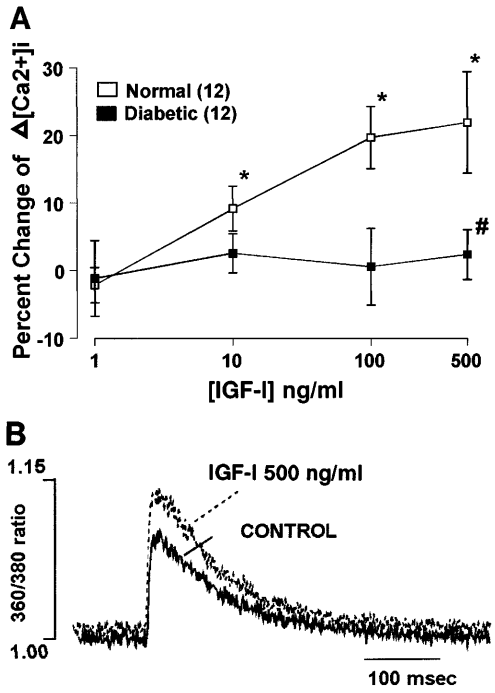


Fig. 6. *A*: effect of IGF-I on intracellular $Ca^{2+}$ transient changes in ventricular myocytes from normal and diabetic hearts. Actual data points in concentration-dependent response curves were obtained by normalizing intracellular $Ca^{2+}$ transient changes (change in fura 2 fluorescence intensity) to respective control value. *B*: typical experiment showing effect of IGF-I (500 ng/ml) on intracellular $Ca^{2+}$ transient before and 5 min after IGF-I addition in a myocyte isolated from a normal heart. $[Ca^{2+}]_i$, intracellular $Ca^{2+}$ concentration. Values are means ± SE, with number of myocytes in parentheses. $^*P <$ 0.05 vs. control; $^{\#}P < 0.05$ vs. normal.

diabetic groups, respectively. The PTA in response to electrical stimulation was 9.5 ± 1.5 and 7.5 ± 1.3% in normal and diabetic groups, respectively. As shown in Fig. 5*A*, the IGF-I-induced potentiation in cell shortening in myocytes from normal hearts was completely abolished by L-NAME, consistent with observations in normal papillary muscles. In diabetic myocytes, the IGF-I-induced decrease of cell shortening was attenuated by L-NAME at low concentration(s), but not at higher concentrations (Fig. 5*B*). As shown in Fig. 5, L-NAME alone had little effect on cell shortening over a duration of 30 min.

*Effect of IGF-I on intracellular $Ca^{2+}$ transients.* To determine whether the differential response to IGF-I in normal and diabetic myocytes was related to alteration of intracellular $Ca^{2+}$ handling, we used fura 2 to estimate changes in $[Ca^{2+}]_i$ in single myocytes. The time course of the fluorescence signal decay (fluorescence decay time, FDT) was evaluated to assess the rate of intracellular $Ca^{2+}$ clearing. Myocytes from both groups exhibited similar baseline fura 2 fluorescence intensity (FFI, representing resting intracellular $Ca^{2+}$ levels). However, diabetic myocytes showed a significantly slower clearing rate (greater FDT value) than normal myocytes, suggesting a dysfunction of intracellular $Ca^{2+}$ resequestration/extrusion. In myocytes from normal animals, acute IGF-I exposure caused a concentration-dependent increase in intracellular $Ca^{2+}$ (ΔFFI = peak FFI − baseline FFI), in a pattern similar to its effect on cell shortening, suggesting that the increase in intracellular free $Ca^{2+}$ in response to electrical stimulation is enhanced by IGF-I (Fig. 6*A*). The threshold of the IGF-I-induced increase of ΔFFI was between 10 and 100 ng/ml. ΔFFI achieved steady state at or before 3 min, and cells recovered completely after a 5-min washout. Also consistent with previous data in papillary muscles and isolated myocytes, IGF-I had no effect on $Ca^{2+}$ transients in myocytes from diabetic animals, suggesting that this lack of effect on $Ca^{2+}$ underlies the lack of an inotropic effect of IGF-I. Neither resting FFI nor FDT was affected by IGF-I (Table 4).

## DISCUSSION

Recent evidence suggests that IGF-I exerts positive effects on cardiac growth and myocardial contraction (11, 32). However, other studies have shown a relative resistance to the action of this hormone in experimental diabetic animals (8, 18, 31). In the present investigation we found that IGF-I exerts positive inotropic effects on papillary muscles and increases the extent of cell shortening in preparations from normal but not diabetic animals. Consistent with the contractile response, IGF-I increased intracellular $Ca^{2+}$ transients in normal but not diabetic myocytes.

Although the myocardial inotropic action of insulin has been extensively examined (26, 34), few studies have assessed the myocardial effect of IGF-I. It has recently been reported that rat hearts exhibit a marked increase in systolic pressure due to an increase of overall $Ca^{2+}$ responsiveness after 4 wk of treatment with IGF-I (32). This is supported by the observation that IGF-I (10 ng/ml) increased papillary muscle PTD and myocyte intracellular $Ca^{2+}$ transients by ~18 and ~26%, respectively (11). In another study, Vetter et al.

Table 4. *Effect of IGF-I on resting intracellular $Ca^{2+}$ transient level (360/380 ratio) and fluorescence decay time in ventricular myocytes from normal and diabetic hearts*

| | | IGF-I | | | |
|---|---|---|---|---|---|
| | Control | 1 ng/ml | 10 ng/ml | 100 ng/ml | 500 ng/ml |
| Resting FFI | | | | | |
| Normal | 1.04 ± 0.03 | 0.99 ± 0.03 | 1.02 ± 0.03 | 1.07 ± 0.04 | 1.08 ± 0.03 |
| Diabetic | 1.01 ± 0.03 | 0.95 ± 0.05 | 1.03 ± 0.04 | 1.04 ± 0.04 | 1.05 ± 0.05 |
| FDT, ms | | | | | |
| Normal | 130 ± 13 | 146 ± 7 | 123 ± 14 | 115 ± 15 | 115 ± 7 |
| Diabetic | 170 ± 17* | 168 ± 13 | 161 ± 11 | 175 ± 10 | 182 ± 13 |

Values are means ± SE of 12 cells/group. FFI, fura 2 fluorescence intensity; FDT, fluorescence decay time. $^*P < 0.05$ vs. normal.

(34) reported that IGF-I increased neonatal myocyte contraction by ~16 and ~22% at physiological concentration of 12 and 25 ng/ml, respectively. Our results are consistent with these observations. In nondiabetic animals, IGF-I disproportionately increased myocardial isometric contraction and myocyte isotonic shortening by ~8 and ~26% at 500 and 100 ng/ml, respectively. This was associated with an ~20% increase in intracellular $Ca^{2+}$ transients at 500 ng/ml of IGF-I. However, no positive action of IGF-I was seen in diabetic myocardium or myocytes. The mechanisms underlying IGF-I-induced inotropic responses and intracellular $Ca^{2+}$ transient changes are not clear, but several possible explanations are worthy of consideration. IGF-I-induced positive inotropic responses in normal animals may be related to an increase in $[Ca^{2+}]_i$. This inotropic effect may have been abrogated by the $Ca^{2+}$ overload (i.e., retention of cytosolic $Ca^{2+}$ due to reduced rate of extrusion) commonly seen in diabetes (7, 23) and reflected by the lengthened FDT in our diabetic myocytes. Decreases in IGF-I message have also been demonstrated in diabetes (1); these altered autocrine/paracrine effects of IGF-I may contribute to the changed contractile response seen in the diabetic state.

Our data suggest that ambient NO levels may modulate IGF-I-induced cardiac inotropic effects. Consititutive NOS (cNOS), as well as inducible NOS, is present in cardiomyocytes (14, 16), and although IGF-I is known to stimulate NO production in various tissue or cell types (32, 34), there has been no direct evidence of IGF-I-induced stimulation of NO or the involvement of specific isoforms of cNOS (neuronal or endothelial NOS) in heart tissue. It is believed that cNOS activation is frequency dependent in ventricular myocytes (15). In this study, papillary muscles and ventricular myocytes were continuously stimulated to contract at 0.5 Hz, but the NOS inhibitor L-NAME alone did not affect PTD in normal or diabetic myocardium. This lack of response to L-NAME may indicate that tonic NO production (under stimulation) is not likely to modulate myocardial contraction and may be similar to the action of NO at physiological concentration under unstimulated conditions (36).

It has been reported that NO induces a biphasic effect on voltage-gated $Ca^{2+}$ currents, including potentiation at lower concentrations and attenuation at higher concentrations (20). Cardiac muscle is known to produce a tonic amount of NO at rest, sufficient to inhibit current through L-type $Ca^{2+}$ channels via a cGMP-dependent mechanism (5, 16). However, when cardiac muscle is stimulated, sarcolemmal $Ca^{2+}$ influx activates sarcoplasmic reticulum $Ca^{2+}$ release and NOS activity. The subsequent increase in NO may lead to a concerted activation of the sarcoplasmic reticulum $Ca^{2+}$ channel via reversible modulation. This effect may override cGMP-induced inhibition of the $Ca^{2+}$ channel (5, 37). By contrast, oxidative stress seen in diabetes may irreversibly activate the channel and thus have deleterious consequences on NO-mediated excitation-contraction coupling (37).

In this study, L-NAME attenuated or abolished the positive inotropic effect of IGF-I in normal myocardium and isolated myocytes. Interestingly, NOS inhibition unmasked a positive inotropic response in diabetic myocardium but not in isolated myocytes. L-NAME is known to inhibit all forms of NOS, but inhibition of inducible NOS requires extensive hydrolysis that may not be achieved during the short incubation time utilized in these in vitro experiments. Thus we expect greater selectivity toward the cNOS present in cardiomyocytes (14). Also, we cannot discount the possibility that a difference in esterase activity, which is required for full L-NAME inhibitory action, may exist in normal vs. diabetic cardiomyocytes (28). However, the results seen with L-NAME in diabetic myocardium probably reflect greater NO production. Recently, Mohan et al. (21) reported that low concentrations of NO and cGMP (responsible, in part, for NO action) induced a positive inotropic effect, whereas high concentrations exerted a negative inotropic response. Thus, as with $[Ca^{2+}]_i$, optimal NO levels may be required for normal myocardial responses to IGF-I. In conjunction with the fact that the activity of heart NOS may be compensatorily increased in diabetes (24), IGF-I may stimulate a greater NO production in diabetic than in normal animals, leading to negative (or no response if tonic NO is not modulating myocardial function) and positive actions. However, L-NAME may shift NO production from high to low or from low to none in diabetic and normal myocardium, respectively, accounting for our observations. The lack of reversal by L-NAME in diabetic myocytes may be related to the fact that the isolated cardiomyocytes may be more easily exposed than myocardial tissue to L-NAME because of the absence of any diffusion barrier. Thus NO production could be completely blocked in myocytes.

In summary, our data demonstrate the presence of resistance to IGF-I-induced myocardial responses in diabetes; this may be mediated by an altered NO level. Abnormal IGF-I level or function has been considered to be an important predisposing factor in diabetes-related vascular disorders and may contribute to the development of cardiac dysfunction. It is thus important to understand the underlying cellular mechanisms associated with the positive inotropic actions of IGF-I and alterations of this response under diabetic conditions. This should provide more background for the potential use of IGF-I as a novel therapeutic agent in the treatment of diabetes mellitus and related cardiac complications.

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Address for reprint requests: R. A. Brown, Dept. of Physiology, Wayne State University School of Medicine, 540 E. Canfield Ave., Detroit, MI 48201.

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