Insulin-Like Growth Factor-1 Exerts Ca\textsuperscript{2+}-Dependent Positive Inotropic Effects in Failing Human Myocardium

Dirk von Lewinski, Kerstin Voß, Swen Hülsmann, Harald Kögler, Burkert Pieske

Abstract—Myocardial generation of insulin-like growth factor-1 (IGF-1) is altered in hypertrophy and heart failure, but there are no reports on acute functional effects of IGF-1 in human cardiac muscle. We examined inotropic responses and signal transduction mechanisms of IGF-1 in human myocardium. Experiments were performed in isolated trabeculae or cardiomyocytes from 46 end-stage failing hearts. The effect of IGF-1 (0.001 to 0.2 \(\mu\)mol/L) on isometric twitch force (37°C, 1 Hz), intracellular Ca\textsuperscript{2+} transients (aequorin method), sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} content (rapid cooling contractures), L-type Ca\textsuperscript{2+} current (whole-cell voltage clamp), and cAMP concentrations was assessed. In addition, the effects of blocking IGF-1 receptors, phosphoinositide-3-kinase (PI-3-kinase), protein kinase C (PKC), or transsarcolemmal Ca\textsuperscript{2+} entry were tested. IGF-1 exerted concentration-dependent positive inotropic effects (twitch force increased to maximally 133±4% of baseline values at 0.1 \(\mu\)mol/L; \(P<0.05\)). The IGF-1 receptor antibody aR3 or the PI-3-kinase inhibitor wortmannin prevented the functional effects. The inotropic response was paralleled by increases in Ca\textsuperscript{2+} transients and SR Ca\textsuperscript{2+} content. IGF-1 (0.1 \(\mu\)mol/L) increased L-type Ca\textsuperscript{2+} current amplitude by 24±7% (\(P<0.05\)). Blockade of SR function did not affect the inotropic response to IGF-1. In contrast, L-type Ca\textsuperscript{2+} channel blockade with diltiazem partially prevented (≈50%) the inotropic response to IGF-1. Inhibition of PKC (GF109203X), Na\textsuperscript{+}-H\textsuperscript{+} exchange (HOE642), or reverse-mode Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange (KB-R7943) reduced the response to IGF-1 by ≈60% to 70%. IGF-1 exerts Ca\textsuperscript{2+}-dependent positive inotropic effects through activation of IGF-1 receptors and a PI-3-kinase–dependent pathway in failing human myocardium. The increased [Ca\textsuperscript{2+}], with IGF-1 originates from both enhanced L-type Ca\textsuperscript{2+} currents and enhanced Na\textsuperscript{+}-H\textsuperscript{+} exchange–dependent reverse-mode Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange. These nongenomic functional effects of IGF-1 may be of clinical relevance. (Circ Res. 2003;92:667–675.)

Key Words: insulin-like growth factor-1 ▪ functional effects ▪ Ca\textsuperscript{2+} handling ▪ heart failure ▪ human myocardium

Insulin-like growth factor-1 (IGF-1), a circulating and locally produced peptide hormone structurally and functionally related to insulin, is synthesized under the control of growth hormone (GH) by various cell types, including cardiac myocytes. IGF-1 stimulates protein synthesis and growth and exerts metabolic as well as antiapoptotic effects in many organs including cardiac muscle. Recently, increased local cardiac IGF-1 release and increased cardiac IGF-1 mRNA production and IGF-1 receptor expression were reported in heart failure animal models and in human cardiac diseases.

Normal activation of the GH-IGF axis is essential for myocardial performance, and GH/IGF-1 deficiency is associated with impaired cardiac function. Chronic GH substitution may induce beneficial long-term effects on myocardial function in GH deficiency and chronic heart failure. Interestingly, acute application of IGF-1 also enhanced circulatory function with increased stroke volume and cardiac output in healthy volunteers and patients with chronic heart failure. However, IGF-1 exerts endothelium-dependent vasodilatory actions, and it remains unclear whether improved hemodynamics result from direct inotropic or peripheral vasodilatory effects of IGF-1.

A limited number of investigations have directly tested the acute functional effects of IGF-1 in mammalian myocardium. In these studies, IGF-1 increased contractility in isolated rat, ferret, or dog myocardium, but the subcellular mechanism of action of IGF-1 remained controversial. No data are available on functional effects of IGF-1 in human cardiac muscle.

Therefore, we directly assessed functional effects and mechanisms of action of IGF-1 in isolated failing human myocardium. Our main finding was that IGF-1 exerts receptor-mediated, Ca\textsuperscript{2+}-dependent positive inotropic effects that are related to a PI-3-kinase–dependent activation of L-type Ca\textsuperscript{2+} currents and Na\textsuperscript{+}-H\textsuperscript{+} exchange.
Materials and Methods

Human Myocardium
Experiments were performed in muscle strips obtained from 46 end-stage failing hearts (from 14 female and 32 male patients). The mean ejection fraction before transplantation was 23.4 ± 0.8%. The study protocol was approved by the local ethics committee. For details, please refer to the expanded Materials and Methods section available in the online data supplement at http://www.circresaha.org.

Muscle Strip Preparation
Small endocardial trabeculae were dissected from the left or right ventricle as previously described, connected to an isometric force transducer, and superfused with bicarbonate-containing Tyrode’s solution (30°C). Muscles were field stimulated at 1 Hz (37°C), and isometric contractions were recorded at optimal preload (Lmax). The functional effects of IGF-1 were assessed by cumulative concentration-response curves (0.001 to 0.1 μmol/L) or by a single, maximally effective concentration of IGF-1 (0.1 μmol/L).

Aequorin Measurements
At steady-state contractile function, the Ca2+-regulated bioluminescent protein aequorin was macroinjected into the quiescent muscle as described previously. Aequorin light emission was detected using a photomultiplier, which was vertically mounted with its cathode just above the glass cuvette containing the muscle.

Rapid Cooling Contractions (RCCs)
RCCs were elicited by a rapid decrease in the temperature of the muscle chamber from 37°C to 1°C by switching from a warm to a cold solution with solenoid pinch valves at the bath inlet as previously described. The resulting cooling contracture is an index for sarcoplasmic reticulum (SR) Ca2+ content.

Patch-Clamp Experiments
Human ventricular myocytes were isolated with collagenase. Myocytes were placed in a 0.5-mL chamber and superfused with Tyrode’s solution (30°C). Currents were recorded using the whole-cell patch-clamp technique. The peak inward current during a depolarization step from -40 to +10 mV was taken as Ifast. At steady-state basal current recordings, IGF-1 (0.1 μmol/L) was added to the superfusate, and the effects of IGF-1 on Ca2+ currents were analyzed.

cAMP Determinations
Intracellular cAMP levels in homogenates of IGF-1– or isoproterenol (ISO)–prestimulated muscle strips were measured by a commercially available [1H]cAMP assay system (Amersham Pharmacia) according to the protocol provided by the manufacturer.

Drugs
Recombinant human IGF-1 (Sigma) or IGF-1 receptor antibody (αIR-3 clone; Oncogene Research Products) was dissolved in Tyrode’s solution and added to the organ bath. Diltiazem (Sigma), HOE642 (Cariporide; a gift of Aventis Pharma), KB-R7943 (Tocris), or wortmannin (Sigma) was added to the organ bath 30 minutes before the experiment.

Statistical Analysis
Data are expressed as mean ± SEM. Differences were compared by paired Student t test or one-way repeated measures ANOVA followed by the Student-Newman-Keuls test when appropriate. Statistical significance was taken as P < 0.05.

Results
Inotropic Effects of IGF-1
IGF-1 (0.1 μmol/L) exerted pronounced positive inotropic effects in isolated human cardiac muscle (Figure 1A, top panel). The inotropic response developed to a stable plateau phase within 3 to 4 minutes without changes in diastolic tension. These functional effects of IGF-1 were mediated via specific IGF-1 receptors; preincubation of a preparation from the same heart with the specific IGF-1 receptor antibody αIR-3 (3.3 μg/mL) for 30 minutes prevented the inotropic response to IGF-1 (bottom panel).

The functional effects of IGF-1 were concentration dependent (Figure 1B). The inotropic response started at 0.01 μmol/L and was maximal at 0.1 μmol/L with an increase in twitch tension to 133.4 ± 4.4% of the baseline value (n = 11, P < 0.05). The EC50 was 0.020 μmol/L (95% confidence interval = 0.015 to 0.027 μmol/L). Diastolic tension and time parameters of contraction and relaxation were not affected by IGF-1. There were no significant differences in inotropic responses to IGF-1 between trabeculae from hearts with dilated or ischemic cardiomyopathy. As can also be seen from Figure 1B, preincubation of muscle strips with the IGF-1 receptor antibody αIR-3 (n = 7) almost completely prevented the inotropic response to IGF-1.

Subcellular Mechanisms of Action of IGF-1
Further experiments were performed in aequorin-loaded muscle strips and compared with the effects of increasing [Ca2+], from 2.5 to 4.0 mmol/L, or of β-adrenoceptor stimulation with ISO (0.1 μmol/L). These concentrations were chosen to achieve inotropic effects comparable to those with IGF-1. Figure 2 shows original tracings of the effects of IGF-1 (0.1 μmol/L, left), and [Ca2+]i (4.0 mmol/L, right) on aequorin signals (upper tracings) and the corresponding isometric twitches (lower tracings). IGF-1 resulted in a similar increase in the amplitude of the aequorin light signal and isometric twitch tension. Likewise, [Ca2+]i, 4.0 mmol/L increased both aequorin light emission and force of contraction to a similar extent. As can be seen from Figure 3 (left), IGF-1 (0.1 μmol/L, n = 6) resulted in a proportional increase in twitch tension and aequorin light emission (by 46% ± 9% and 51% ± 12% of the basal value, respectively; P < 0.05). To further elucidate the IGF-10–dependent signal transduction pathways, wortmannin (0.1 μmol/L) was used to block the phosphoinositide-3-kinase (PI-3-kinase) in aequorin-loaded muscle strips. Preincubation with wortmannin did not affect basal contractile force or relaxation (Table 1), but almost completely prevented both the increase in twitch force and Ca2+ transient sents after IGF-1 (Figure 3, right).

Inotropic effects may be brought about by cAMP-dependent or -independent mechanisms. cAMP-dependent interventions are usually associated with enhanced relaxation and an overproportional increase in intracellular Ca2+ transients. Table 2 summarizes the effects of IGF-1, Ca2+, and ISO on twitch force, aequorin light emission, and twitch and Ca2+ transient decay parameters. All inotropic interventions yielded comparable inotropic responses. However, while the relative increase in force (F) and aequorin light emission (L) was similar with IGF-1 or [Ca2+]i, 4.0 mmol/L (ΔF/ΔL was 1.02 ± 0.38 and 1.03 ± 0.12, respectively), the increase in aequorin light emission relative to force was significantly higher with ISO (ΔF/ΔL was 0.56 ± 0.11). Although this may indicate that ISO (in contrast to IGF-1) overproportionally
increases intracellular Ca\(^{2+}\) transients relative to force, these relationships have been obtained only at one time point and must be interpreted with caution. Furthermore, relaxation time of the isometric twitch and decay time of the Ca\(^{2+}\) transient were significantly abbreviated with ISO but remained unchanged with IGF-1 and [Ca\(^{2+}\)]\(_o\) 4.0 mmol/L (Table 2). These experiments suggest that IGF-1 exerts its positive inotropic effect by a cAMP-independent increase in intracellular Ca\(^{2+}\) transients.

To support this hypothesis, we directly measured cAMP concentrations in trabeculae under control conditions and after stimulation with IGF-1 or ISO. Under control conditions...
tions, cAMP levels were 470±158 pg/mL and did not increase after preincubation with IGF-1 (616±126 pg/mL, NS). In contrast, after preincubation with ISO (0.1 μmol/L), cAMP concentrations were increased to 1208±247 pg/mL ($P<0.05$ versus control or IGF-1).

**Effects of IGF-1 on SR Ca$^{2+}$ Handling**

We performed rapid-cooling experiments to directly assess the effects of IGF-1 on SR Ca$^{2+}$ content. Figure 4A shows a representative original recording. The upper tracing reflects temperature near the surface of the muscle, and the lower tracing, twitch force. At steady-state isometric contractions, the muscle was cooled to ≈1°C, and a stable cooling contracture as an index for SR Ca$^{2+}$ content developed. On rewarming, the muscle completely relaxed, and the experimental protocol was repeated after addition of IGF-1. IGF-1 increased both isometric twitch tension and the amplitude of the cooling contracture. The average data are presented in Figure 4B (left). IGF-1 (0.1 μmol/L) significantly increased twitch force and RCCs by 38.8±9.9% and 27.6±4.2%, NS) or Ca$^{2+}$ preparations from 6 hearts. Wortmannin did not affect basal force ($−6.1±4.2$, NS) or Ca$^{2+}$ transient amplitude ($−10.2±7.4$, NS). $^*P<0.05$ vs basal value before IGF-1 administration. % of base (ordinate) signifies percentage change from basal value of developed force or aequorin light emission before IGF-1 administration.

**Table 1. Influence of Pharmacological Blockers on Basal Force and Relaxation**

<table>
<thead>
<tr>
<th>Blocker</th>
<th>ΔF, %</th>
<th>ΔRT$_{50}$, ms</th>
<th>ΔRT$_{50}$, ms</th>
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<tbody>
<tr>
<td>Wortmannin (0.1 μmol/L)</td>
<td>$−6±4$</td>
<td>$+3±3$</td>
<td>$0±9$</td>
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<tr>
<td>CPA/Rya (20 and 1 μmol/L)</td>
<td>$−42±11^*$</td>
<td>$+25±4^*$</td>
<td>$+26±4^*$</td>
</tr>
<tr>
<td>Diltiazem (5 μmol/L)</td>
<td>$−53±11^*$</td>
<td>$−13±6$</td>
<td>$+5±10$</td>
</tr>
<tr>
<td>HOE642 (3 μmol/L)</td>
<td>$−10±3^*$</td>
<td>$−5±4$</td>
<td>$−6±4$</td>
</tr>
<tr>
<td>KB-R7943 (5 μmol/L)</td>
<td>$−42±7^*$</td>
<td>$−1±3$</td>
<td>$−1±3$</td>
</tr>
<tr>
<td>GF109203X (1 μmol/L)</td>
<td>$−1±4$</td>
<td>$+4±3$</td>
<td>$+3±3$</td>
</tr>
</tbody>
</table>

$\Delta F$ indicates change in force of contraction; $\Delta R_{T50}$, change in relaxation time to 50% of peak tension; $\Delta R_{T50}$, change in relaxation time to 10% of peak tension; CPA, cyclopiazonic acid; and Rya, ryanodine. $^*P<0.05$. 

**Effects of IGF-1 on Transsarcolemmal Ca$^{2+}$ Influx**

We further assessed the contribution of transsarcolemmal Ca$^{2+}$ influx to inotropic responses to IGF-1. Major routes of

**Figure 3.** Left, Effects of IGF-1 (0.1 μmol/L) on twitch force (filled bar) and aequorin light emission (open bar). Average data from 6 preparations from 6 hearts. Right, Same protocol as above, but after preincubation with 0.1 μmol/L wortmannin. Average data from 6 preparations from 6 hearts. Wortmannin did not affect basal force ($−6.1±4.2$, NS) or Ca$^{2+}$ transient amplitude ($−10.2±7.4$, NS). $^*P<0.05$ vs basal value before IGF-1 administration. % of base (ordinate) signifies percentage change from basal value of developed force or aequorin light emission before IGF-1 administration.

**Figure 4A.** Changes in twitch tension and aequorin light emission before and after preincubation with IGF-1. 

**Figure 4B (left).** IGF-1 (0.1 μmol/L) significantly increased twitch force and RCCs by 38.8±9.3% and 27.6±4.2%, NS) or Ca$^{2+}$ preparations from 6 hearts. Wortmannin did not affect basal force ($−6.1±4.2$, NS) or Ca$^{2+}$ transient amplitude ($−10.2±7.4$, NS). $^*P<0.05$ vs basal value before IGF-1 administration. % of base (ordinate) signifies percentage change from basal value of developed force or aequorin light emission before IGF-1 administration.
Ca\textsuperscript{2+} entry in human myocardium are through L-type Ca\textsuperscript{2+} channels or reverse-mode Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange.\textsuperscript{21,24} We first tested whether IGF-1 affects transsarcolemmal Ca\textsuperscript{2+} influx through L-type Ca\textsuperscript{2+} channels. For this purpose, we analyzed L-type Ca\textsuperscript{2+} currents by whole-cell voltage-clamp techniques in isolated cardiomyocytes. Figure 5A shows typical current recordings during depolarization steps from \(-40\) to \(+10\) mV. It can be seen that the amplitude of the Ca\textsuperscript{2+} current increases, while the decay kinetics remain unchanged. Similar experiments were performed in a total of 5 ventricular myocytes (Figure 5B). In these experiments, the basal current amplitude induced by the voltage step from \(-40\) to \(+10\) mV was \(2.54 \pm 0.63\) nA. IGF-1 (0.1 \textmu mol/L) significantly increased \(I_{\text{Ca,L}}\) amplitude to \(124 \pm 7\%\) of the basal value.

In a separate set of experiments in trabeculae, the effects of preincubating muscle strips with the L-type Ca\textsuperscript{2+} channel antagonist diltiazem (5 \textmu mol/L) on the inotropic response to IGF-1 was investigated. At this concentration, diltiazem exerted a pronounced negative inotropic effect (Table 1). Applied at contractile steady-state conditions, the inotropic response to IGF-1 was significantly reduced in the presence of the Ca\textsuperscript{2+} channel blocker (Figure 6). These experiments suggest that the increase in intracellular Ca\textsuperscript{2+} transients and twitch force with IGF-1 are partly mediated by enhanced Ca\textsuperscript{2+} entry through L-type Ca\textsuperscript{2+} channels.

<table>
<thead>
<tr>
<th></th>
<th>ΔF, %</th>
<th>ΔL, %</th>
<th>ΔF/ΔL</th>
<th>ΔRT\textsubscript{95}, ms</th>
<th>ΔRL\textsubscript{80}, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-1 (0.1 \textmu mol/L)</td>
<td>46 \pm 9*</td>
<td>51 \pm 12*</td>
<td>1.02 \pm 0.38</td>
<td>(-2 \pm 20)</td>
<td>(-13 \pm 14)</td>
</tr>
<tr>
<td>[Ca\textsuperscript{2+}]\textsubscript{o} (4.0 mmol/L)</td>
<td>51 \pm 4*</td>
<td>49 \pm 5*</td>
<td>1.03 \pm 0.12</td>
<td>(+4 \pm 8)</td>
<td>+1 \pm 4</td>
</tr>
<tr>
<td>ISO (0.1 \textmu mol/L)</td>
<td>63 \pm 12*</td>
<td>153 \pm 55*</td>
<td>0.56 \pm 0.11</td>
<td>(-62 \pm 13*)</td>
<td>(-31 \pm 12*)</td>
</tr>
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</table>

ΔF indicates change in force of contraction; ΔL, change in aequorin light signal; ΔRT\textsubscript{95}, change in relaxation time to 5% of peak tension; and ΔRL\textsubscript{80}, change in decay time to 20% of peak light emission. *P<0.05.

\(\Delta F\) indicates change in force of contraction; \(\Delta L\), change in aequorin light signal; \(\Delta RT_{95}\), change in relaxation time to 5% of peak tension; and \(\Delta RL_{80}\), change in decay time to 20% of peak light emission. *P<0.05.

Table 2. Influence of IGF-1, [Ca\textsuperscript{2+}]\textsubscript{o}, and Isoproterenol on Force and Aequorin Light Amplitude and on Decay Time of Force and Aequorin Light

Figure 4. A, Original recording of rapid cooling experiment. Top part depicts bath temperature; bottom part, twitch force. Upon cooling, a stable cooling contracture develops. With rewarming, the muscle completely relaxes after a brief rewarming spike. At steady-state conditions, IGF-1 (0.1 \textmu mol/L) is added, and the experiment is repeated. B, Left, Average values from 7 preparations from 7 hearts for experiments as shown in Figure 4A. Right, Additional experiments were performed in 7 preparations from 6 hearts after blocking SR function with cyclopiazonic acid (CPA) and ryanodine. Data are compared with basal values before IGF-1 administration. This intervention completely prevented RCCs but not the increase in twitch force with IGF-1. % of base (ordinate) signifies percentage change from the basal value of developed force or RCC amplitude before IGF-1 administration.
Intracellular Ca\textsuperscript{2+} accumulation may also be modulated by the Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger. This electrogenic ion transporter extrudes Ca\textsuperscript{2+} for Na\textsuperscript{+} influx in its forward mode but may also work in its reverse mode, resulting in Ca\textsuperscript{2+} influx during depolarization.\textsuperscript{24} The latter may be favored by increases in [Na\textsuperscript{+}]. Interestingly, recent experiments demonstrated an IGF-1–mediated stimulation of the sarcolemmal Na\textsuperscript{+}-H\textsuperscript{+} exchanger in vascular smooth muscle cells.\textsuperscript{25} In the present study, preincubation of muscle strips with the Na\textsuperscript{+}-H\textsuperscript{+} exchange inhibitor HOE642 (3 μmol/L) only slightly affected basal force (Table 1) but resulted in a significant reduction in the maximal inotropic response to IGF-1 (by 60±15%; \(P<0.05\); Figure 6). In a further set of experiments, the effects of the reverse-mode Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange inhibitor KB-R7943 (5 μmol/L) were tested. KB-R7943 exerted a significant depressant effect on basal force of contraction (Table 1). Applied at steady-state contractile conditions, the positive inotropic effect of IGF-1 was reduced by 70±9% (\(P<0.05\); Figure 6) in the presence of KB-R7943. These data indicate that the inotropic response to IGF-1 is related to enhanced transsarcolemmal Ca\textsuperscript{2+} entry through both L-type Ca\textsuperscript{2+} channels and reverse-mode Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange. The latter may be secondary to an IGF-1–mediated activation of the Na\textsuperscript{+}-H\textsuperscript{+} exchanger with subsequent [Na\textsuperscript{+}] accumulation. Because both Na\textsuperscript{+}-H\textsuperscript{+} exchange and L-type Ca\textsuperscript{2+} channels may be activated through protein kinase C (PKC),\textsuperscript{26,27} we preincubated muscle preparations with the PKC inhibitor GF109203X (n=4). PKC inhibition did not affect basal contractility (Table 1), but it resulted in a significant reduction in the inotropic response to IGF-1 by 69±4% (\(P<0.05\)).

**Discussion**

This is the first report on functional effects of IGF-1 in isolated human myocardium. The results show that (1) IGF-1...
exerts a concentration-dependent positive inotropic effect that is almost completely prevented by blocking IGF-1 receptors or PI-3-kinase, (2) the inotropic effect is related to a cAMP-independent increase in intracellular Ca\(^{2+}\) transients, and (3) IGF-1 increases L-type Ca\(^{2+}\) currents and activates Na\(^{-}\)-H\(^{+}\) exchange and reverse-mode Na\(^{+}\)-Ca\(^{2+}\) exchange.

**Acute and Long-Term Effects of IGF-1 in the Cardiovascular System**

IGF-1 exerts acute hemodynamic as well as long-term genomic effects in the cardiovascular system. Genomic effects include induction of muscle growth, hypertrophy, and antapoptotic signaling.\(^1\) Experimental and clinical studies indicate that long-term application of GH or IGF-1 may have beneficial effects in heart failure.\(^3\),\(^10\),\(^28\)

In addition, acute application of IGF-1 to healthy volunteers or to patients with heart failure increases cardiac output,\(^12\),\(^13\) but the underlying mechanisms for these hemodynamic effects remain unclear. Noninvasive studies demonstrated endothelium-dependent vasodilatory actions of IGF-1 in humans, which could account for improved hemodynamics on infusion of IGF-1.\(^14\),\(^15\) IGF-1 exerted direct positive inotropic effects in isolated mammalian cardiac muscle,\(^16\)–\(^18\) and inotropic effects of IGF-1 could contribute to enhanced circulatory function in humans. However, no data on direct functional effects of IGF-1 in the human heart are available.

**Direct Functional Effects of IGF-1 in Isolated Human Myocardium**

We observed a \(-35\%\) increase in isometric twitch force at 0.1 \(\mu\)mol/L IGF-1 without changes in relaxation parameters or diastolic function. This inotropic response to IGF-1 amounts to 30\% to 40\% of the maximal positive inotropic effects of \(\beta\)-adrenoceptor stimulation in failing human myocardium.\(^29\)

Therefore, the acute functional effects of IGF-1 are substantial and of potential clinical relevance. The magnitude of the inotropic responses to IGF-1 in this study is comparable to the effects previously reported in isolated neonatal\(^16\) and adult\(^30\) rat myocytes, canine myocytes,\(^18\) and ferret trabeculae.\(^17\)

The inotropic response to IGF-1 in the present study started at a concentration of 0.01 \(\mu\)mol/L (75.8 ng/mL) and was maximal at a concentration of 0.1 \(\mu\)mol/L (758 ng/mL). These concentrations are identical to those in isolated ferret muscle\(^17\) and comparable to experiments in isolated rat and dog myocytes.\(^19\),\(^29\) In humans, IGF-1 plasma levels do not reach such high values.\(^31\) However, increases in local cardiac IGF-1 peptide content and upregulation of IGF-1 receptors as well as increased local cardiac IGF-1 release have been observed in myocardium from animal models of pressure or volume overload or ischemic cardiomyopathy.\(^5\),\(^7\) In addition, myocardial hypertrophy was associated with a cardiaco-specific increase in IGF-1 mRNA expression and peptide generation.\(^6\) Therefore, IGF-1 exerts its effects on cardiac myocytes not only in an endocrine but also an autocrine/paracrine fashion within the heart, and IGF-1 concentrations at the level of the myocytes may be substantially higher than in the systemic circulation.

**Signal Transduction Pathways of IGF-1 in Human Myocardium**

IGF-1 may bind to both types of IGF receptors and to insulin receptors, but the IGF-1 receptor, a heterotetrameric protein with intrinsic tyrosine kinase activity, is most abundant in adult myocardium.\(^31\),\(^32\) In the present study, IGF-1 effects were almost completely prevented by preincubation with the selective IGF-1 receptor antibody aIR-3. This demonstrates the role of specific IGF receptors of the subtype 1 in the functional effects of IGF-1 in human myocardium.

Activation of several signal transduction pathways after IGF-1 receptor stimulation were described.\(^33\) One of these pathways involves phosphorylation of insulin-receptor substrate with consecutive activation of PI-3-kinase.\(^1\) The PI-3-kinase–dependent pathway mediates antiapoptotic signaling and trophic effects via activation of Akt, a serine/threonine protein kinase,\(^34\) and promotes glycoen synthesis through phosphorylation of glycost synhase kinase 3.\(^34\) In the present study, preincubation of human ventricular muscle with the selective PI-3-kinase inhibitor wortmannin prevented the IGF-1–dependent inotropic effect and the increase in Ca\(^{2+}\) transients. This demonstrates that PI-3-kinase activation is a key event in the signal transduction network ultimately resulting in functional effects. The involvement of PI-3-kinase activation in the inotropic response to IGF-1 is consistent with a previous report in rat heart.\(^18\) On the other hand, PI-3-kinase inhibition was described to modify \(\beta\)\(-\)adrenoceptor signaling in adult rat myocardium enabling \(\beta\)\(-\)adrenoceptors to induc phospholamban phosphorylation and increase contractility and Ca\(^{2+}\) transients.\(^35\) This finding may point to distinct pathways of PI-3-kinase signaling depending on the trigger and the consecutive activation of PI-3-kinase isoforms.

In addition, PKC inhibition partly prevented the inotropic response to IGF-1 in the present study, and PKC activation occurred downstream from PI-3-kinase activation in mammalian myocardium.\(^36\) However, the exact signaling cascade responsible for functional effects of IGF-1 remains to be elucidated. For example, PI3-kinase–dependent Akt activation is a key element in the protection of cardiomyocytes from cell death,\(^33\) but the contribution of Akt to functional effects of IGF-1 is unclear.

**Influence of IGF-1 on Intracellular Ca\(^{2+}\) Handling**

The functional responses to IGF-1 were accompanied by an increase in intracellular Ca\(^{2+}\) transients in the present study. An increase in [Ca\(^{2+}\)]\(_i\) as the underlying mechanism for the inotropic effect of IGF-1 was also demonstrated in rat myocytes\(^18\),\(^30\) but not in rat whole-heart preparations.\(^17\) The mechanisms involved in the increase in Ca\(^{2+}\) on acute administration of IGF-1 are unknown. Principally, a rise in [Ca\(^{2+}\)]\(_i\), may result from cAMP-dependent (eg, \(\beta\)-adrenoceptor stimulation) or cAMP-independent mechanisms.\(^23\),\(^29\) The present data strongly suggest that the functional effects of IGF-1 result from cAMP-independent mechanisms for several reasons, as follows: (1) \(\beta\)-adrenoceptor stimulation with ISO, but not IGF-1, enhanced relaxation rate and decay of the Ca\(^{2+}\) transients; (2) IGF-1 increased force and aequorin light emission similar to [Ca\(^{2+}\)]\(_i\), whereas the
increase in aequorin light emission was much more pronounced with ISO; and (3) cAMP levels in isolated human myocardium did not change with IGF-1 but were largely increased with ISO.

Inotropic compounds may also directly modulate the responsiveness of the myofilaments for Ca\(^{2+}\). Previous reports on the effects of IGF-1 on myofilament Ca\(^{2+}\) sensitivity in mammalian myocardium are contradictory. Cittadini et al\(^{17}\) reported an IGF-1–dependent increase in myofilament Ca\(^{2+}\) sensitivity, but Kinugawa et al\(^{18}\) could not detect changes in the myofilament responsiveness in rat myocytes. A Ca\(^{2+}\)-sensitizing mode of action typically results in little or no increase in intracellular Ca\(^{2+}\) transients, prolonged relaxation, and diastolic dysfunction.\(^{23,29}\) Given that Ca\(^{2+}\) transients increased, and relaxation time as well as diastolic function remained unchanged in the present study, sensitization of the myofilaments does not appear to be a major mode of action of IGF-1 in human myocardium.

We extended the study of intracellular Ca\(^{2+}\) handling and assessed the effects of IGF-1 on SR function and Ca\(^{2+}\) content. RCCs revealed that the inotropic response to IGF-1 was associated with increased SR Ca\(^{2+}\) content. However, pharmacological blockade of SR function did not prevent the inotropic response to IGF-1. Therefore, the increase in SR Ca\(^{2+}\) load after IGF-1 stimulation probably results from increased Ca\(^{2+}\) availability within the myocytes and not from direct IGF-1 stimulation of SR Ca\(^{2+}\) uptake. This is also supported by the unchanged RCC2/RCC1 ratio in the present study.

**Influence of IGF-1 on Transsarcolemmal Ca\(^{2+}\) Influx**

Recently, Solem and Thomas\(^{37}\) demonstrated a substantial increase in dihydropyridine-sensitive Ca\(^{2+}\) channel activity in cardiac myocytes on exposure to IGF-1. In fact, in the present study, L-type Ca\(^{2+}\) currents significantly increased in the presence of IGF-1 in isolated human cardiomyocytes. Furthermore, diltiazem partly prevented the inotropic response to IGF-1. In addition to L-type Ca\(^{2+}\) channel–mediated effects, inotropic responses to IGF-1 could be reduced to a similar extent by inhibition of the Na\(^{+}\)-H\(^{+}\) exchanger with HOE642 or by the reverse mode of the Na\(^{+}\)-Ca\(^{2+}\) exchanger with KB-R7943. Therefore, activation of Na\(^{+}\)-H\(^{+}\) exchange contributes to the acute effects of IGF-1 in human myocardium, possibly via enhanced Ca\(^{2+}\) entry through [Na\(^{+}\)]-dependent activation of the reverse-mode Na\(^{+}\)-Ca\(^{2+}\) exchange function.\(^{24}\) This is supported by a recent study in rat smooth muscle cells in which IGF-1 increased [Ca\(^{2+}\)], through activation of Na\(^{+}\)-H\(^{+}\) exchange.\(^{25}\)

Taken together, in myocardium from failing human hearts, the functional effects of IGF-1 are mediated by PI-3-kinase–dependent increases in intracellular Ca\(^{2+}\) transients. The data indicate that IGF-1–induced activation of L-type Ca\(^{2+}\) channels, as well as Na\(^{+}\)-H\(^{+}\) exchange, followed by enhanced reverse-mode Na\(^{+}\)-Ca\(^{2+}\) exchange, contributes to the increase in force and [Ca\(^{2+}\)]. Neither increases in cAMP nor altered Ca\(^{2+}\) responsiveness of the myofilaments appears to contribute significantly to the acute inotropic responses to IGF-1. Our data indicate that the potential pathophysiological and clinical relevance of IGF-1–dependent signaling in human heart failure deserves further investigation.

**Limitations of the Study**

One limitation of the study is that isolated muscle strip preparations produce less contractile force than could be expected from the undamaged, intact organ. However, the basal developed force of the muscles used in this study (22 ± 2 mN/mm\(^2\)) is comparable or even higher as reported in previous studies using failing human myocardium.\(^{19,20,38}\) Nevertheless, muscle strips may undergo damage during transport and preparation, and this may affect the results. A further limitation of the study is that aequorin light signals have not been converted to [Ca\(^{2+}\)]. This in itself is not a problem, but it largely precludes direct comparison between different inotropic interventions. Although validated for human cardiac muscle,\(^{20}\) rapid cooling may not release all Ca\(^{2+}\) stored within the SR.\(^{39}\) In addition, RCCs are an indirect measure of SR Ca\(^{2+}\) content, and subcellular changes, such as a potential IGF-1–induced increase in myofilament responsiveness to Ca\(^{2+}\), may also result in increased RCCs. The postulated IGF-1–dependent activation of Na\(^{+}\)-H\(^{+}\) exchange could result in increased pH, resulting in enhanced myofilament Ca\(^{2+}\) responsiveness. However, changes in pH are unlikely to occur in the presence of bicarbonate-containing physiological buffer solutions\(^{40}\) and could not be detected in preliminary (n = 3) experiments using BCECF in human preparations. Furthermore, pharmacological blockade of NHE1 and, more likely, reverse-mode Na\(^{+}\)-Ca\(^{2+}\) exchange may not be fully specific.\(^{41,42}\) Therefore, these experiments indicate, but do not prove, involvement of sodium-dependent exchangers in the inotropic response to IGF-1.

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**References**


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