short-term administration of insulin-like growth factor I (IGF-1) does not induce myocardial IGF-1 resistance

Jun Ren

Department of Pharmacology, Physiology and Therapeutics, University of North Dakota School of Medicine, Grand Forks, ND 58203, USA

Summary The advent of recombinant technology has revealed attractive therapeutic profile of insulin-like growth factor I (IGF-1) in the treatment of chronic cardiovascular diseases such as diabetes and heart failure. However, the safety and potential adverse effect of IGF-1 have not been well defined. This study was designed to evaluate the impact of short-term IGF-1 administration on myocardial contractile function. Adult rats were given recombinant human IGF-1 (3 mg/kg/d, s.c.) for 8 weeks. Mechanical properties were evaluated in left-ventricular papillary muscles using a force-transducer. Myocardial contractile properties analyzed included peak tension development (PTD), time-to-peak tension (TPT), time-to-90% relaxation (RT$_{90}$), and maximal velocity of tension development/decline ($\pm$VT). Short-term IGF-1 treatment enhanced the plasma IGF-1 level but had no effect on body and organ weights. The myocardium from IGF-1-treated rats exhibited enhanced PTD associated with similar TPT, RT$_{90}$, and $\pm$VT compared to the control group. IGF-1-treated myocardium exhibits an enhanced PTD-Ca$^{2+}$ response and a better intracellular Ca$^{2+}$ replenishing ability at the low stimulus frequencies. Acute application of IGF-1 (1–500 ng/ml) elicited a comparable concentration-dependent increase in PTD in myocardium from both control and the IGF-1-treated groups. Acute IGF-1 application had no effect on $\pm$VT, TPT, and RT$_{90}$ in either group tested. Pretreatment with the nitric oxide synthase inhibitor N$\omega$-nitro-L-arginine methyl ester blunted the IGF-1-induced positive response in myocardium from both control and IGF-1-treated groups. These results suggest that short-term IGF-1 treatment is unlikely to induce IGF-1 resistance in myocardial contractile function.

Key words: IGF-1, papillary muscles, contraction

INTRODUCTION

Insulin-like growth factor I (IGF-1), a peptide growth factor structurally and functionally similar to insulin, is synthesized by various cell types including cardiomyocytes and acts as an autocrine/paracrine factor. Recent advances in recombinant technology have made it possible for IGF-1 to be used in a variety of diseases such as diabetes, heart failure, osteoporosis, and muscle atrophy. Administration of recombinant human IGF-1 increases energy expenditure and lipid oxidation, reduces insulin and growth hormone secre-
tion, and increases insulin sensitivity. These metabolic properties of IGF-1 have implicated its clinical usefulness in the treatment of insulin resistance, dyslipidemia, or diseases with low circulating IGF-1 levels (e.g., diabetes and senescence), which are commonly associated with high cardiovascular morbidity and mortality. IGF-1 has been demonstrated to facilitate glucose transport and metabolism, and improve glucose control in both types of diabetes. In addition, accumulating evidence has suggested that IGF-1 has specific cardiovascular effects in addition to its well-defined growth promoting and metabolic effects. IGF-1 may directly promote myofibril development and cardiac contractility. Although the ability for IGF-1 to repair and/or improve cardiac function is intriguing, it is still difficult to discern between a direct contraction enhancing effect and an indirect growth promoting of recombinant IGF-1 therapy.

Elevated circulating IGF-1 levels may lead to undesirable effects. High doses of IGF-1 administration (120 µg/kg bid) were reported to be associated with intolerable side effects in type 2 diabetic patients. It has been suggested that the adverse effect of IGF-1 may be related to its treatment-induced alteration in IGF-1 and IGFBP level, which regulates the activity of IGF-1 de novo. Therefore, the aim of the present study was to examine the effect of short-term recombinant IGF-1 treatment on the myocardial contractile response to IGF-1, to understand the relationship between IGF-1 treatment and subsequent IGF-1 responsiveness. Since the ultimate goal of IGF-1 therapy in any cardiac disease would be to restore or improve the ventricular pump function, this study evaluated the mechanical properties of left ventricular papillary muscles as an index for cardiac pumping function.

MATERIALS AND METHODS

Animal IGF-1 administration

The experimental procedures described here were conducted in compliance with the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health (Publication No. 85-23, 1996) and were approved by our Institutional Animal Care and Use Committee. Briefly, adult male Sprague-Dawley rats (~150 g) were randomly divided into IGF-1 receiving (Genentech, South San Francisco, 3 mg/kg/d, s.c.) and control (received saline) groups. Short-term IGF-1 administration lasted for eight weeks before the animals were sacrificed. At the time of sacrifice, plasma was collected and extracted with formic acid acetone (to remove interfering IGFBP) before total IGF-1 levels were determined using an ELISA commercial kit from Diagnostic System Laboratory (Webster, TX).

Ventricular papillary muscle isolation and measurement of isometric tension

Upon completion of the eight-week period, animals were anesthetized with ketamine/xylazine (5:3, 1.32 mg/kg). Hearts were rapidly excised and immersed in oxygenated (95% O2-5% CO2) Tyrode’s solution (mM: KCl 5.4, NaCl 136.9, NaHCO3 11.9, MgCl2 0.50, CaCl2 2.70, NaH2PO4 0.45, and glucose 5.6, pH 7.4) at 37°C. Left-ventricular papillary muscles were dissected and mounted in a temperature-controlled (30°C) bath superfused with Tyrode’s solution. The sub-physiological temperature (30°C) was used to maintain a longer duration (4–6h) of steady-state contraction. Preparations were allowed to equilibrate for 90 min while electrically driven at 0.5Hz, to establish baseline isometric tension development. Length-tension curves were constructed for each preparation and the peak tension development (PTD) was recorded at approximately 90% of Lmax using a force transducer (Grass, FT 03). The parameters measured included PTD (normalized to muscle weight), time-to-peak tension (TPT), time-to-90% relaxation (RT90), and maximal velocity of tension development/decline (±VT). Following the equilibration period, muscles were exposed to IGF-1 cumulatively. Recovery was continuously monitored after removal of the drug from the bath. The maximal response of IGF-1 or insulin was reached within 5 min of exposure and remained steady for more than 30 min. Therefore all measurements were taken at the 5 min exposure time. In some studies, the papillary muscles were pre-incubated with the nitric oxide (NO) synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME, 100 µM) for 20 min prior to IGF-1 addition, to evaluate the role of NO in the IGF-1-induced response.

Data analysis

Data are presented as means ± SEM. The difference between means for each variable was evaluated with one-way analysis of variance (ANOVA) or t test, where appropriate. A Dunnett’s test was used for post hoc analysis. A p value < 0.05 was considered statistically significant.

RESULTS

Effect of IGF-1 administration on baseline myocardial contractile function

Short-term daily administration of IGF-1 (8 weeks) significantly increased the plasma IGF-1 level while little effect was seen in body and organ weight.
Table 1 General features of experimental animals

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Body Wt (g)</th>
<th>Heart Wt (g)</th>
<th>Heart Wt/Body Wt (mg/g)</th>
<th>Liver Wt/Body Wt (mg/g)</th>
<th>Kidney Wt/Body Wt (mg/g)</th>
<th>Plasma IGF-1 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (7)</td>
<td>367 ± 31</td>
<td>1.12 ± 0.06</td>
<td>3.1 ± 0.1</td>
<td>41.7 ± 2.0</td>
<td>8.5 ± 0.4</td>
<td>102 ± 10</td>
</tr>
<tr>
<td>IGF-1 (5)</td>
<td>366 ± 7</td>
<td>1.18 ± 0.04</td>
<td>3.2 ± 0.1</td>
<td>34.0 ± 1.6</td>
<td>10.0 ± 1.2</td>
<td>429 ± 31</td>
</tr>
</tbody>
</table>

Wt: weight. Mean ± SEM, * p < 0.05 vs. control, (n): number of animals.

Fig. 1 Influence of short-term IGF-1 administration (3 mg/kg/d) on myocardial contractile function in left ventricular papillary muscles from rat hearts. (A) weight of left-ventricular papillary muscles; (B) peak tension development normalized to papillary muscle weight (g/g); (C) time-to-peak tension development (TPT); (D) time-to-90% relaxation (RT90); (E) maximal velocity of tension development (+VT); (F) maximal velocity of tension decline (−VT). Mean ± SEM, n = 10 papillary muscles per group. * p < 0.05 vs. the control group.

As shown in Fig. 1, short-term IGF-1 administration did not significantly affect papillary muscle mass. The PTD, normalized to respective papillary muscle mass, was significantly elevated in myocardium from the IGF-1 treated group compared with the control group. However, the duration and
maximal velocity of contraction and relaxation (TPT, RT90, ±VT) were all similar in myocardium between the control and IGF-1 groups. These data suggest that short-term administration of IGF-1 promotes cardiac contractility without a noticeable adverse effect on mechanical function, at least in the current experimental setting.

**Influence of IGF-1 administration on PTD in response to increased bath Ca²⁺ concentration**

IGF-1 has been shown to increase the myofilament Ca²⁺ sensitivity.₁₅,₁₆ To evaluate the influence of short-term IGF-1 administration on myocardial inotropic responsiveness to changes in the extracellular Ca²⁺ concentration, the bath Ca²⁺ concentration was increased from 0.5 to 10 mM (our normal bath Ca²⁺ concentration is 2.7 mM). As shown in Fig. 2A, elevated extracellular Ca²⁺ level elicited positive inotropic responses of PTD in both groups, which peaked at 3.0–4.0 mM of bath Ca²⁺. Interestingly, PTD obtained at all bath Ca²⁺ concentrations was significantly greater in myocardium from the IGF-1-treated group compared with the control group. This result indicated that IGF-1 administration enhances the myofilament responsiveness to Ca²⁺, consistent with previous reports.₁₅,₁₆

**Influence of IGF-1 administration on PTD in response to increased stimulus frequency**

Rat hearts normally contract at very high frequencies (>300 beat/min), whereas our baseline studies were conducted at 0.5 Hz. To look for possible derangement of mechanical properties, we increased the stimulating frequency from 0.1 to 5 Hz (300 beat/min) and recorded the steady-state PTD. Papillary muscles were initially stimulated to contract at 0.5 Hz for 5 min to ensure steady-state before commencing the frequency study. All the recordings were normalized to PTD obtained at 0.1 Hz of the same muscle. Fig. 2B shows a negative staircase in PTD with increasing stimulating frequency in preparations from both groups. However, short-term IGF-1 administration alleviated the reduction in PTD at 0.3 and 0.5 Hz compared to the control group, indicating that the replenishment of intracellular Ca²⁺ storage may be better preserved at lower stimulus frequencies. TPT, RT90, and ±VT were comparable between the control and IGF-1 groups throughout the spectrum of stimulus frequencies tested (data not shown).

**Effect of IGF-1 administration on myocardial contractile response to IGF-1 and insulin**

Acute application of IGF-1 (1–500 ng/ml) caused a concentration-dependent increase in PTD in myocardium from both control and the IGF-1 treatment groups, with the thresholds between 1–10 ng/ml, and 10–100 ng/ml, respectively (Fig. 3A). The effect of IGF-1 on myocardial contraction was reversible upon washout. Since NO has been implicated to play a role in the IGF-1-induced cardiac contractile response,¹¹ the myocardial contractile response to IGF-1 was re-examined in the presence of the NOS inhibitor Nω-nitro-L-arginine methyl ester (L-NAME, 100 μM). Consistent with our previous findings,¹¹ L-NAME abolished the IGF-1-induced positive response in myocardium from both control and IGF-1 treatment.
groups (Fig. 3B). Insulin (1–500 nM) failed to exert any myocardial contractile response in either control or the IGF-1 administration group (Fig. 3C). Lastly, acute IGF-1 application did not significantly affect the velocity or duration of contraction and relaxation (±VT, TPT and RT90) and such patterns of response were not affected by short-term IGF-1 administration (Fig. 4). These data suggest that short-term IGF-1 treatment is unlikely to induce myocardial IGF-1 resistance or to improve the insulin responsiveness in myocardial contractile function.

DISCUSSION

IGF-1 has been implicated to play a role in the treatment of certain chronic disorders such as growth hormone resistance, heart failure, and diabetes. However, prolonged IGF-1 treatment and/or elevated plasma IGF-1 levels have been associated with certain adverse effects ranging from dizziness, fatigue, palpitations, and flushing to dyspnea or even transient cerebral dysfunction. The IGF-1 dosage used in the current study (3 mg/kg/d) was much higher than the doses commonly used clinically (< 200 µg/kg/d). Therefore, findings from the current study suggest that short-term recombinant IGF-1 therapy improves myocardial contractility and Ca\(^{2+}\) sensitivity without affecting the myocardial responsiveness to IGF-1 or insulin. These data may be useful to understand the relationship between IGF-1 treatment and subsequent IGF-1 responsiveness and to evaluate the safety of short-term IGF-1 therapy clinically for certain cardiac complications.

In our study, short-term IGF-1 therapy elevated the plasma IGF-1 level whereas it did not affect body, organ, and papillary muscle weight. This suggests that the short-term treatment regime is not necessarily associated with the “growth-promoting” effect of the peptide, which often accompanies long-term administration of IGF-1. As a critical mediator in the growth of muscle and other tissues, both systemic administration and transgenic overexpression of IGF-1 lead to increased muscle protein content and reduced protein degradation. It may be speculated that the lack of effect of short-term IGF-1 therapy on organ weight (or protein synthesis although it was not determined in our study) may be related to an inability to maintain sufficiently high local IGF-1 levels for a prolonged period of time. In contrast, it has been hypothesized that the mechanism by which IGF-1 increases cardiac function is, at least in part, beyond its general anabolic effects through a direct inotropic effect on target ventricular myocytes. This notion is supported by our current findings of improved myocardial contractility (PTD), Ca\(^{2+}\) responsiveness, and intracellular Ca\(^{2+}\) replenishing capacity after short-term IGF-1 therapy, consistent with the previous report. Further study should be warranted to elucidate the mechanisms of action responsible for the improved cardiac function independent of its “growth-promoting properties” following IGF-1 therapy.

IGF-1 is an endogenous regulator of myocardial function. Its cardiac contractile response is characterized by rapid onset, long lasting with the modest magnitude compared with other endogenous substances, therefore suggesting that the peptide may
exert its regulatory effects on myocardial contractile function in a time frame over minutes to hours, sensitizing the myofilaments to rises in $[\text{Ca}^{2+}]_{i}$ induced by more potent but short-lived neurohumoral factors. The enhanced PTD obtained throughout 0.5–10 mM bath Ca$^{2+}$ in the IGF-1 group suggests a higher myofilament Ca$^{2+}$ responsiveness following the short-term peptide treatment, consistent with the improved PTD capability in the IGF-1 treatment group. The enhanced myofilament Ca$^{2+}$ sensitivity, in conjunction with a faster ability to replenish intracellular Ca$^{2+}$ pool as suggested by the PTD-frequency response, facilitates the myocardial intracellular Ca$^{2+}$ cycling. The most important finding from the current study is that the IGF-1-induced positive myocardial contractile response was not affected by short-term IGF-1 therapy. IGF-1 is a key cardiac regulating hormone and its cardiac resistance, which has been found in several disease states including diabetes and hypertension, may be used as a marker for the development of cardiac dysfunctions. Maintained IGF-1 responsiveness following the short-term IGF-1 therapy suggests that the IGF-1 receptor and post-receptor signaling system(s) may have been preserved under the short-term high circulating IGF-1 environment. An earlier study from our laboratory revealed that the IGF-1-induced cardiac contractile response may be mediated via ambient NO levels. In the current study, the NOS inhibitor L-NAME blunted the IGF-1-induced contractile response in myocardium from both groups, indicating that the involvement of NO in the IGF-1-induced cardiac contractile response may not be affected by short-term IGF-1 administration. Lastly, the inability of IGF-1 treatment to alter the myocardial response to insulin suggests that the metabolic and inotropic effect of IGF-1 on insulin sensitivity may be completely independent of each other, although either mechanism may contribute to the preservation of heart function in disease status where insulin resistance is present.

Taken together, this study demonstrated that short-term IGF-1 administration improves cardiac contractility and myofilament Ca$^{2+}$ responsiveness without eliciting any adverse effect on IGF-1-induced myocardial mechanical responses. Considering the attractive therapeutic profile of IGF-1 in chronic cardiac conditions such as heart failure and diabetes, the inability to develop any myocardial IGF-1 resistance should ensure normal IGF-1 regulation in cardiac function.
pumping function. It will also provide essential information in elucidating the clinical safety of short-term IGF-1 therapy. However, it remains to be determined whether the preserved mechanical function seen after short-term IGF-1 treatment will prevail following prolonged recombinant IGF-1 therapy.

ACKNOWLEDGMENTS

This work was supported in part by North Dakota Max Baer Heart Fund. The author gratefully acknowledges Genentech Inc., (South San Francisco, CA) for providing the human recombinant IGF-1 and Dr. Fariba Roughead from Grand Forks Human Nutrition Center for plasma IGF-1 assay.

REFERENCES