Influence of Age on Contractile Response to Insulin-Like Growth Factor 1 in Ventricular Myocytes From Spontaneously Hypertensive Rats

Jun Ren, LeQuishia Jefferson, James R. Sowers, Ricardo A. Brown

Abstract—Evidence suggests a pathophysiological role of insulin-like growth factor 1 (IGF-1) in hypertension. Cardiac function is altered with advanced age, similar to hypertension. Accordingly, the effects of IGF-1 on cardiac myocyte shortening and intracellular Ca$^{2+}$ were evaluated in hypertension at different ages. Ventricular myocytes were isolated from Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR), aged 12 and 36 weeks. Mechanical and intracellular Ca$^{2+}$ properties were examined by edge-detection and fluorescence microscopy. At 12 weeks, IGF-1 (1 to 500 ng/mL) increased peak twitch amplitude (PTA) and FFI changes (∆FFI) in a dose-dependent manner in WKY myocytes, with maximal increases of 27.5% and 35.2%, respectively. However, IGF-1 failed to exert any action on PTA and ∆FFI in the age-matched SHR myocytes. Interestingly, at 36 weeks, IGF-1 failed to exert any response in WKY myocytes but depressed both PTA and ∆FFI in a dose-dependent manner in SHR myocytes, with maximal inhibitions of 40.5% and 16.1%, respectively. Myocytes from SHR or 36-week WKY were less sensitive to norepinephrine (1 μmol/L) and KCl (30 mmol/L). Pretreatment with nitric oxide synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME, 100 μmol/L) did not alter the IGF-1–induced response in 12-week WKY myocytes but unmasked a positive action in 12-week SHR and 36-week WKY myocytes. L-NAME also significantly attenuated IGF-1–induced depression in 36-week SHR myocytes. In addition, the Ca$^{2+}$ channel opener Bay K8644 (1 μmol/L) abolished IGF-1–induced cardiac depression in 36-week SHR myocytes. Collectively, these results suggest that the IGF-1–induced cardiac contractile response was reduced with advanced age as well as with hypertension. Alterations in nitric oxide and intracellular Ca$^{2+}$ modulation may underlie, in part, the resistance to IGF-1 in hypertension and advanced age.

Key Words: insulin growth factor, hypertension, essential, aging, myocytes, calcium

Hypertension, a major risk factor for stroke and myocardial infarction, often leads to cardiac hypertrophy and electromechanical abnormalities such as prolonged action potential and contraction duration. Clinical and experimental investigations have shown an association between insulin resistance, hyperinsulinemia, and hypertension. However, the exact nature of this relation remains to be established. Biophysical and biochemical evidence has also suggested that myocellular structure and function alter with advanced age in a manner similar to hypertension, although aging itself may not be sufficient to induce cardiac hypertrophic response and hypertension. It is believed that individual myocyte function may be preserved with developed age but that myocyte apoptosis and replacement by extracellular matrix may contribute substantially to the decrement in active cardiac contractile function in aging. Recent evidence has also suggested that insulin resistance plays a key role in cardiovascular abnormalities in aging, although the underlying mechanisms have not been elucidated.

Insulin-like growth factor (IGF-1), a 70–amino acid basic peptide, is an essential growth factor for cellular proliferation and differentiation. It can be synthesized and act as an autocrine/paracrine factor, exerting both inotropic and growth effects in the heart. Unlike insulin, 95% of the circulating IGF-1 is bound to IGF-1 binding proteins (IGFBPs), mainly IGFBP-3. These IGFBPs are believed to mediate the actions of IGF-1 in a cell- and tissue-specific manner. IGF-1 has been shown to mediate multiple physiological as well as pathophysiological responses in the cardiovascular system. IGF-1 stimulates protein synthesis and participates in the initiation and development of left ventricular hypertrophy. IGF-1 has also been reported to increase inositol 1,4,5-tris-phosphate levels, activate tyrosine kinase, tyrosine kinase phosphatase, phosphatidylinositol-3 kinase, and protein kinase C, and enhance myocardial contraction and intracellular Ca$^{2+}$ level or sensitivity, suggesting a possible role in normal cardiac mechanical function. How-
ever, recent studies have revealed a positive correlation between circulating IGF-1 level and hypertension. Both IGF-1 mRNA and protein levels have been reported to be elevated in the heart in parallel with the development of hypertension. With the use of rat heart papillary muscles, we recently observed that hypertension and advanced age significantly attenuated IGF-1–induced myocardial force-generating capacity, indicating that IGF-1 may play a role in the altered myocardial function under hypertension and/or advanced age. To date, the underlying mechanism of altered myocardial response has not been elaborated.

The aims of the present study were to investigate the cardiac contractile response of IGF-1 in single ventricular myocytes isolated from hypertensive and normotensive rats at a young age (12 weeks) and relatively old age (36 weeks). We also sought to study the mechanism(s) of action underlying IGF-1–induced cardiac contractile action. Spontaneously hypertensive rats (SHR), a model for human essential hypertension, and age-matched normotensive Wistar-Kyoto rats (WKY) were used. The SHR develops left ventricular hypertrophy in response to sustained elevated arterial blood pressure and total peripheral resistance. This model is also characterized by defects in insulin/IGF-1 actions similar to those observed in essential hypertension. Development of ventricular dysfunction also appears to increase with age or the duration of hypertension. Thus, the present study was designed to evaluate the effect of IGF-1 on cardiac contractility/Ca2+ metabolism in WKY and SHR at an advanced age (36 weeks).

Methods

Animals

The experimental procedures were approved by the Wayne State University Animal Investigation Committee. To determine whether hypertension and advanced age affect the cardiac contractile response to IGF-1, adult male WKY and SHR were obtained from Taconic Farms (Germantown, NY) at 4 weeks of age and maintained through 12 or 36 weeks of age. The animals were individually housed in a temperature-controlled room under a 12-hour light/dark illumination cycle and were allowed standard rat chow and tap water ad libitum. Systolic blood pressures and body weights were obtained on a weekly basis by using the tail-cuff method and a standard laboratory balance, respectively.

Cell Isolation

At the end of the experimental period, single ventricular myocytes were enzymatically dissociated from the rat hearts by the method described. Isolated myocytes were plated on glass coverslips and maintained in a serum-free medium at 37°C. Mechanical properties remained relatively stable for 12 to 24 hours. Only rod-shaped myocytes with clear edges were selected for study.

Intracellular Fluorescence Measurement

A separate cohort of myocytes was loaded with fura 2-AM (0.5 μmol/L) for 15 minutes at 30°C, and fluorescence measurements were recorded with a dual-excitation fluorescence photomultiplier system as described. Fluorescence emissions were detected between 480 and 520 nm after first illuminating cells at 360 nm for 0.5 seconds and then at 380 nm for the duration of the recording protocol. The 360-nm excitation scan was repeated at the end of the protocol, and an interpolated signal was calculated and used to calculate the 360/380 ratio as fura 2 fluorescence intensity (FFI).

Experimental Protocols

Myocytes were allowed to contract at a frequency of 0.5 Hz over 5 minutes to ensure steady state before superfusion with IGF-1. In some studies, the nitric oxide synthase (NOS) inhibitor N-nitro-L-arginine methyl ester (L-NAME, 100 μmol/L, Sigma Chemical Co) was incubated with the muscles for 15 minutes before IGF-1 addition. Myocytes with rundown >10% in PTA over the first 5 minutes were not studied further. The maximal response of IGF-1 was achieved within 4 minutes and remained steady for >20 minutes. Therefore, all the measurements were taken after a 5-minute exposure to this hormone.

Data Analysis

Data are presented as mean±SEM. Differences between and within groups were evaluated by 2-way ANOVA with repeated measures (SYSTAT). A Tukey test was used as a follow-up for the multiple comparison.
comparisons. To determine significant differences in the repeated measures factor (concentration of IGF-1), the “within-subjects” mean square (MS) error and df error terms from the parent ANOVA were used. To determine significant differences between strains at a given concentration of IGF-1, the “between-subjects” MS error and df error terms from the parent ANOVA were used. Statistical significance was considered to be $P<0.05$.

Results

General Features of WKY and SHR

The impact of hypertension and advanced age on blood pressure and body, liver, and kidney sizes are shown in Table 1. As expected, SHR exhibited a significantly elevated systolic blood pressure and a lower body weight compared with their WKY counterparts throughout the course of the study. Advanced age did not alter the blood pressure. Hypertension at an early stage (12 weeks) had no effect on the size of liver and kidney. However, sustained hypertension led to hepatomegaly and renal hypertrophy in SHR compared with their age-matched WKY counterparts. The size of liver and kidney were actually reduced with advanced age, which might be due to an increased body weight.

Baseline Mechanical Properties of Ventricular Myocytes

Sustained hypertension tended to lead to cardiac hypertrophy. Ventricular myocytes isolated from 36- but not 12-week SHR hearts exhibited considerably larger dimension (either cell length or surface area) than did WKY myocytes. Advanced age alone had little effect on cell dimension. Neither cell length nor surface area was affected by IGF-1 exposure (data not shown). Myocytes isolated from 12-week animals displayed a similar extent of shortening capacity, as indicated by PTA. However, PTA was significantly greater in myocytes from 36-week SHR compared with those from age-matched WKY. The enhanced ability to shorten in older SHR myocytes was associated with increased maximal velocities of shortening and relengthening ($\pm dL/dt$). Interestingly, the myocyte shortening capacity decreased with advanced age. The shortening and relengthening durations (TPS and TR90) were not affected by early stage of hypertension and were also reduced with advanced age. Moreover, compared with the age-matched WKY myocytes, myocytes from older SHR exhibited a prolonged TPS along with a normal TR90 (Table 2).
Effects of IGF-1 on Myocyte Shortening in WKY and SHR Myocytes

Representative traces depicting typical effects of IGF-1 on cell shortening are shown in Figure 1. IGF-1 (500 ng/mL) significantly increased PTA in myocytes from 12-week WKY but not SHR (Figure 1A). At the end of a 5-minute exposure to this concentration of IGF-1, PTA was increased by 28.4% and 3.7%, with little effect on TPS and TR90, in WKY and SHR myocytes, respectively. IGF-1 (1 to 500 ng/mL) caused a concentration-dependent increase in PTA in myocytes from young WKY but not SHR, with a maximal response of 27.5% (Figure 1C). The threshold was between 1 and 10 ng/mL. Higher concentrations of IGF-1 (to 1000 ng/mL) did not induce further increase of PTA. By contrast, representative traces in Figure 1B show that IGF-1 (500 ng/mL) significantly depressed PTA in myocytes from 36-week SHR but not WKY. IGF-1 caused a concentration-dependent depression in PTA in myocytes from older SHR but not WKY, with a maximal inhibition of 40.5% (Figure 1C). The threshold of the depressive action was also between 1 and 10 ng/mL. These data indicate that the IGF-1–induced cardiac contractile response is altered by hypertension and advanced age.

For comparison with IGF-1, the effect of norepinephrine (NE, 1 μmol/L) and KCl (30 mmol/L) on myocyte shortening was also examined in these myocytes. Both agonists significantly increased PTA to a much greater extent in myocytes from both WKY and SHR hearts. The responsiveness to both agonists decreased or showed a tendency to decrease in the hypertensive state or with advanced age. Finally, insulin (100 nmol/mL) exerted little action on myocyte shortening. These data underscore the differential actions of IGF-1 compared with other contractile agonists (Figure 2).

Effects of IGF-1 on Shortening and Relengthening Duration and Velocity

As mentioned, the baseline TPS and TR90 were not different between myocytes from 12-week WKY and SHR; however, TPS was significantly longer in 36-week SHR myocytes than in age-matched WKY myocytes, whereas TR90 values were not significantly different. Also, 36-week SHR myocytes exhibited significantly greater velocity of both myocyte shortening and relengthening (Table 2). IGF-1 (1 to 500 ng/mL) did not exert any effects on the TPS and TR90 in any cell group studied (data not shown). Consistent with its effect on PTA, IGF-1 (500 ng/mL) increased ±dL/dt in 12-week WKY myocytes (339±51/−360±66 versus baseline value 248±24/−223±17 μm/ms, P<0.05) and showed a tendency to decrease ±dL/dt in 36-week SHR myocytes (318±65/−311±56 versus baseline value 378±34/−355±40 μm/ms, P>0.05).

Effect of IGF-1 on Myocyte Shortening in the Presence of L-NAME and Bay K8644

To explore one possible mechanism of action of IGF-1, the effect of IGF-1 was reexamined in the presence of either the NOS inhibitor L-NAME (100 μmol/L) or voltage-dependent Ca2+ channel opener Bay K8644 (1 μmol/L). L-NAME alone did not modify PTA at the dose used over a duration of >30 minutes. Bay K8644 increased PTA by ~110% in all myocyte groups. As shown in Figure 3, L-NAME did not affect the IGF-1–induced increase in cell shortening in 12-week WKY myocytes, whereas L-NAME unmasked a positive contractile response in 12-week SHR myocytes. Interestingly, the pattern of L-NAME–induced response was not preserved with advanced age. L-NAME unmasked a positive response in 36-week WKY myocytes that was similar to that in 12-week SHR myocytes. Moreover, the IGF-1–induced depression in 36-week SHR myocytes was significantly attenuated by L-NAME pretreatment. The Ca2+ channel opener Bay K8644 also blocked IGF-1–induced depressive action in 36-week SHR myocytes, whereas it had little action in 36-week WKY myocytes (Figure 3B). These data suggested that a substantial tonic nitric oxide (NO) production may exist in hypertension and/or advanced age and that this NO production may lead to a decreased sarcolemmal Ca2+ channel activity.

Effect of IGF-1 on Intracellular Ca2+ Transients

To determine whether the altered response to IGF-1 in hypertension and/or advanced age is related to changes in intracellular Ca2+ level, we used fura 2 to estimate changes in [Ca2+]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. Compared with myocytes from WKY groups, myocytes from the respective SHR groups exhibited elevated baseline FFI (representing resting intracellular Ca\(^{2+}\) level). Advanced age also elevated the resting intracellular Ca\(^{2+}\) level (Table 3). These data indicated a potential Ca\(^{2+}\) overload associated with hypertension and/or advanced age. Representative traces in Figure 4A depict typical IGF-1–induced responses on the electrically stimulated increase of intracellular Ca\(^{2+}\) (ΔFFI=peak FFI−baseline FFI) in WKY and SHR myocytes. Acute IGF-1 (1 to 500 ng/mL) exposure exerted a concentration-dependent increase of ΔFFI in 12-week WKY but not SHR myocytes, in a pattern similar to its effect on PTA. The maximal increase was 35.2% at 500 ng/mL, and the threshold of action was between 1 and 10 ng/mL. However, IGF-1 (1 to 500 ng/mL) caused a concentration-dependent inhibition in ΔFFI in 36-week SHR myocytes but elicited little response in 36-week WKY myocytes. The maximal inhibition was 16.1%, and the threshold of response was between 10 and 100 ng/mL (Figure 4). All the intracellular Ca\(^{2+}\) responses achieved steady state at or before 4 minutes and recovered after a 5-minute washout. These results indicated that the IGF-1–induced cardiac mechanical responses were likely due to changes of intracellular free Ca\(^{2+}\). However, the disproportional inhibition between PTA and ΔFFI in 36-week SHR may indicate a shift in intracellular Ca\(^{2+}\) sensitivity. Neither resting FFI nor FDT was affected by IGF-1 in any of the groups studied (data not shown).

**Discussion**

Pressure-overload ventricular hypertrophy is often characterized by “early” and “late” changes in the expression of certain genes and contractile protein isoforms. Previous studies have demonstrated early hypertrophic responses, including a transient expression of proto-oncogene products that regulates cardiac growth and differentiation, as well as a shift in the major forms of contractile proteins, such as α- and β-myosin heavy chain proteins. Increased atrial natriuretic polypeptide and collagen type I/III have also been reported in the early stage of cardiac hypertrophy.\(^{27}\) During the transition from cardiac hypertrophy to failure, late changes such as marked increase in collagen, fibronectin mRNA, and transforming growth factor-β1 levels can be detected, indicating that the expression of specific extracellular matrix genes may contribute to fibrosis, tissue stiffness, and impaired function.\(^{5,28}\) Recent evidence of the coordinate enhancement of IGF-1 and IGF-1 receptor expression in the early hypertensive stage suggests that IGF-1 may participate in a cascade of events that link pressure stimuli to cellular hypertrophy and, eventually, to cardiac mechanical dysfunction.\(^{11,22}\) Hypertension and/or advanced age are believed to be closely associated with insulin resistance and hyperinsulinemia.\(^{2,6}\) Although evidence has suggested that insulin/IGF-1, per se, may not play a role in hypertension, insulin/IGF-1 resistance, under some circumstances, may contribute to the pathogenesis of hypertension (or similar cardiovascular abnormalities with

**TABLE 3. General Intracellular Ca\(^{2+}\) Transient Characteristics of Ventricular Myocytes From 12- and 36-wk WKY and SHR**

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>Resting FFI, 360/380</th>
<th>Peak FFI, 360/380</th>
<th>FDT, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-wk WKY</td>
<td>0.82±0.01</td>
<td>0.94±0.02</td>
<td>202±7</td>
</tr>
<tr>
<td>12-wk SHR</td>
<td>1.02±0.05*</td>
<td>1.12±0.05*</td>
<td>247±18*</td>
</tr>
<tr>
<td>36-wk WKY</td>
<td>0.94±0.04†</td>
<td>1.06±0.05</td>
<td>192±16</td>
</tr>
<tr>
<td>36-wk SHR</td>
<td>1.13±0.05*</td>
<td>1.33±0.05*</td>
<td>190±22</td>
</tr>
</tbody>
</table>

*Values are mean±SEM (n=12 to 16 per group).

*P<0.05 vs respective WKY group.

†P<0.05 vs 12-wk WKY group.

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Figure 3. A, Effects of L-NAME on IGF-1–induced response on cell shortening in 12-week WKY (left) or SHR (right) myocytes. B, Effects of L-NAME and Bay K8644 on IGF-1–induced response on cell shortening in 36-week WKY (left) and SHR (right) myocytes. Cells were pretreated with either L-NAME (100 μmol/L) or Bay K8644 (1 μmol/L) for 15 and 5 minutes, respectively, before application of IGF-1 (100 to 500 ng/mL). Data are presented as percent change in PTA from the respective control value. The number of myocytes is given in parentheses. *P<0.05 vs control; #P<0.05 vs WKY group.
advanced age), in a manner similar to that found with other pathophysiological factors, such as salt and its effect on the renin-angiotensin system.\textsuperscript{2,24} Resistance in myocardial contractile response to IGF-1, a cardiac autocrine/paracrine peptide that closely resembles insulin, also exists in hypertension and advanced age.\textsuperscript{23} The present investigation lends further support to the notion of IGF-1 resistance in hypertension and/or advanced age. In the present study, sustained hypertension but not advanced age triggered myocyte hypertrophy, although both caused myocyte mechanical dysfunction. IGF-1 exerted positive cardiac contractile responses in myocytes from young WKY but not the age-matched SHR group. However, this IGF-1–induced positive response was abolished at an older age and reversed to a negative inotropic response in myocytes from 36-week SHR animals.

IGF-1 has been demonstrated to improve cardiac function by enhancing myocardial contractility and decreasing peripheral vascular resistance under both physiological and pathological conditions.\textsuperscript{12,16–19,29,30} Although IGF-1 may enhance cardiac contractility through an increase in contractile protein synthesis, several intracellular signaling pathways related to IGF-1 have also been implicated, including tyrosine kinase, tyrosine kinase phosphatase, phosphatidylinositol-3 kinase, and protein kinase C.\textsuperscript{2,14–16} Activation of one or more of these intracellular signaling pathways may be directly related to the elevation of intracellular Ca\textsuperscript{2+} in concert with myocardial contraction and myocyte shortening.\textsuperscript{17–19} Results from the present study have demonstrated similar effects in young WKY animals. However, this positive response was not seen in age-matched SHR or older WKY groups. Older SHR myocytes even revealed the depressive effect of IGF-1 on PTA and DFFI. The attenuated or reversed responsiveness to IGF-1 in hypertension and/or advanced age may be related to the reduced myocardial IGF-1 receptors or postreceptor responses under these conditions. However, there are several reports of an increased IGF-1 receptor mRNA and protein levels in various models of hypertension, including human essential hypertension.\textsuperscript{9,21,22,34,35} An elevation in vascular wall stress is believed to be an important predisposing factor for the elevated gene expression of IGF-1, IGF-1 receptor, and other growth factor receptors in the cardiovascular system.\textsuperscript{35} On the other hand, high levels of circulating IGF-1/insulin, commonly seen in hypertension, may result in a downregulation of IGF-1/insulin receptor binding or effectiveness.\textsuperscript{21} The precise mechanism accounting for the dissociation between the increased IGF-1 receptor expression and the reduced mechanical re-

**Figure 4.** Concentration-dependent response of IGF-1 (1 to 500 ng/mL) on intracellular Ca\textsuperscript{2+} transient changes (ΔFFI) in ventricular myocytes from WKY (■) or SHR (■) hearts of both 12-week (left) and 36-week (right) age groups. Insets at the top are the results of typical experiments showing the effect of IGF-1 (500 ng/mL) on intracellular Ca\textsuperscript{2+} transients in myocytes isolated from 12-week WKY (left) and 36-week SHR (right) hearts. *P < 0.05 vs control; P<0.05 vs WKY group.
Response in hypertension is still not clear. The differential response between IGF-1 and insulin seen in the present study may be related to an altered expression of IGF-1/insulin hybrid receptors found in insulin-resistant states such as hypertension.21

NO has been implicated in the endogenous control of myocardial contractility. Constitutive NOS is present in cardiac myocytes and has been demonstrated to be regulated by a β-adrenergic signaling pathway.36 Constitutive NOS activation is frequency dependent in ventricular myocytes,37 as NO production increases when myocytes are continuously stimulated to contract. IGF-1 is known to stimulate NO production in various tissue or cell types.38,39 Data from the present study indicate that NO may be involved in the IGF-1–induced cardiac contractile response. In young animals, NOS inhibition failed to modify IGF-1–induced positive contractile response in WKY myocytes, whereas it unmasked a positive contractile response in SHR myocytes. However, in the older groups, NOS inhibition unmasked a positive response in WKY myocytes and blocked the IGF-1–induced depressive action in SHR myocytes. These findings indicate altered NO mechanisms in hypertension and/or advanced age.

The fact that Bay K8644 blocked the IGF-1–induced myocardial depression in SHR myocytes may also support the hypothesis of cardiac NO overproduction in hypertension and/or advanced age.40,41 because NO is known to inhibit the voltage-dependent Ca2+ channel, and this inhibition leads to a negative inotropic response.42,43 However, the methods used in the present study were unable to detect any disparity in NO production under hypertension and/or advanced age. Direct measurement of NOS activity in cardiac myocytes is needed to better understand the mechanism underneath the resistance to IGF-1 in these pathophysiological states. Last, the reduced response to IGF-1 and other agonists such as NE may also be associated with the existence of an intracellular Ca2+ overload or diminished β-adrenergic activity in hypertension and/or advanced age.3

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References


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