An analysis of the effects of stretch on IGF-I secretion from rat ventricular fibroblasts

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Hu BS, Landeen LK, Aroonsakool N, Giles WR. An analysis of the effects of stretch on IGF-I secretion from rat ventricular fibroblasts. Am J Physiol Heart Circ Physiol 293: H677–H683, 2007. First published March 30, 2007; doi:10.1152/ajpheart.01413.2006.—Mechanical force can induce a number of fundamental short- and long-term responses in myocardium. These include alterations in ECM, activation of cell-signaling pathways, altered gene regulation, changes in cell proliferation and growth, and secretion of a number of peptides and growth factors. It is now known that a number of these autocrine/paracrine factors are secreted from both cardiomyocytes and ventricular cardiac fibroblasts (CFb) in response to stretch. One such substance is IGF-I. IGF-I is an important autocrine/paracrine factor that can regulate physiological or pathophysiological responses, such as hypertrophy. In this study, we addressed the possible effects of mechanical perturbation, biaxial strain, on IGF-I secretion from adult rat CFb. CFb were subjected to either static stretch (3–10%) or cyclic stretch (10%; 0.1–1 Hz) over a 24-h period. IGF-I secretion from CFb in response to selected stretch paradigms was examined using ELISA to measure IGF-I concentrations in conditioned media. Static stretch did not result in any measurable modulation of IGF-I secretion from CFb. However, cyclic stretch significantly increased IGF-I secretion from CFb in a frequency- and time-dependent manner compared with nonstretched controls. This stretch-induced increase in secretion was relatively insensitive to changes in extracellular [Ca\(^{2+}\)] or to block of L-type Ca\(^{2+}\) channels. In contrast, thapsigargin, an inhibitor of sarcoplasmic reticulum Ca\(^{2+}\) ATPase, remarkably decreased stretch-induced IGF-I secretion from CFb. We further show that IGF-I can upregulate mRNA expression of atrial natriuretic peptide in myocytes. In summary, cyclic stretch can significantly increase IGF-I secretion from CFb, and this effect is dependent on a thapsigargin-sensitive pool of intracellular [Ca\(^{2+}\)].

cardiac fibroblasts; insulin-like growth factor I; thapsigargin; biaxial strain; calcium

CARDIAC FIBROBLASTS (CFb) are essential for extracellular matrix formation and, thus, can regulate diastolic function. CFb can also modulate cardiac growth and development. In addition to releasing collagen, these cells can synthesize and release autocrine/paracrine factors that are important for myocardial remodeling (16, 29, 49). CFb, along with all other cells of the heart, are subjected to dynamic mechanical changes as a consequence of excitation-contraction coupling and pressure force generation during each cardiac cycle. This dynamic cyclical stress may regulate normal cardiac function, and it is thought to contribute to pathophysiological responses. Recent evidence suggests that, although CFb do not contribute to active phasic contraction, they may function as mechanosensors to monitor these mechanical changes (22, 29). In response to stretch, CFb can initiate activation of ERK and JNK pathways, enhance G protein activation, and modulate intracellular Ca\(^{2+}\)-dependent signaling pathways. It is well known that stretched CFb exhibit changes in growth factor secretion [e.g., transforming growth factor-\(\beta\)1 (TGF-\(\beta\)1)] and alter collagen production, which secondarily changes the organization of the extracellular matrix (1, 22, 38, 45). It is also known that stretch-induced responses can be transmitted to cardiac myocytes by paracrine signaling (45). Based on those studies, it has been suggested that endothelin-1 and TGF-\(\beta\)1 from conditioned media of stretched CFb can act as autocrine/paracrine mediators of stretch-induced hypertrophy.

Insulin-like growth factor I (IGF-I) is a single-chain polypeptide protein hormone. Structurally, it is homologous to insulin. Although its synthesis has been studied most extensively in the liver, IGF-I is synthesized in almost all tissues (11, 51). This locally produced IGF-I is now recognized as an important source for autocrine/paracrine signaling pathways that can regulate growth, proliferation, and survival. A recent study demonstrated that locally expressed cardiac IGF-I mRNA and protein can lead to physiological cardiomyocyte hypertrophy (30). It has also been reported that IGF-I produced by CFb can act as a local stimulant for cardiac hypertrophy and remodeling (15). IGF-I can also participate in a number of important pathological processes that are involved in cardiovascular disease. In vivo studies have shown that IGF-I expression is upregulated in the heart during hypertrophic responses and following myocardial infarction (10, 18). Exogenous administration of IGF-I can result in increased protein synthesis in cardiac myocytes (13, 19) and augment both proliferation and collagen secretion in CFb (6, 44). Furthermore, recent studies have suggested a cardioprotective role for IGF-I. IGF-I can reduce myocyte apoptosis in the setting of myocardial infarction, in ischemia-reperfusion injury, and in cultured cardiac myocytes (14). However, despite the growing evidence of the important roles that IGF-I may play, the way in which the mechanical activity of the heart can stimulate IGF-I secretion is not known.

Mechanical stretch can stimulate secretion of autocrine/paracrine factors in a number of different cell systems. For example, in response to mechanical stimuli, cardiac myocytes release ANG II and endothelin-1, and CFb release TGF-\(\beta\)1 and adrenomedullin (43, 45). Although IGF-I can modulate a variety of cellular responses (36), the function of this growth factor in physiological and pathological states is unclear because the regulation of its release is not completely understood. Neonatal CFb are known to secrete IGF-I constitutively in part...
IGF-I secretion from stretched ventricular fibroblasts

Materials and methods

Isolation and culture of CFb and myocytes. CFb were isolated from the ventricles of adult (4–7 wk, 150–250 g) male Sprague-Dawley rats under isoflurane anesthesia in accordance with protocols approved by the Institutional Animal Care and Use Committee and adhered to the “Guidelines for the Care and Use of Laboratory Animals” (1996). Isolated hearts were placed into Ca2+-free Tyrode solution (8) containing 30 mM taurine (pH 7.2–7.4) and were Langendorff perfused through the aorta. After retrograde perfusion with oxygenated 37°C Tyrode solution for 5 min, each heart was perfused with Tyrode solution containing 1 mg/ml collagenase type 2 (Worthington Biochemical; Lakewood, NJ) or 125 μg/ml Liberase Blendzyme 4 (Roche, Indianapolis, IN) or 125 M CaCl2 for 10–15 min at a flow rate of 5–10 ml/min. The digested ventricles were filtered through a 100-μm diameter nylon mesh. Myocytes were removed by centrifugation (50 g for 2 min) and stored for paracrine experiments. The CFb-containing supernatant was pelleted (750 g for 5 min), resuspended, pelleted again over a 4% BSA density gradient, and plated in Dulbecco’s modified Eagle’s medium (DMEM; Gibco/Invitrogen, Carlsbad, CA), supplemented with 10% FBS (Gibco/Invitrogen) for 25 min in a humidified atmosphere of 5% CO2–95% atmospheric air at 37°C. Nonadherent cells were removed by aspiration. All CFb preparations were grown to confluence and subsequently passed at 1:3 split ratios using trypsin-EDTA (Gibco/Invitrogen). All CFb used in these experiments were passed two to three times and used at 12–14 days after isolation. Immunostaining was performed on selected cell populations to assess purity and to confirm that these adult rat CFb cultures did not contain significant numbers of other contaminating cell types. Myocytes were acclimated to 1.8 mM lactic acid to confirm purity and to confirm that IGF-I, at concentrations comparable with those that have been measured in mechanically stretched CFb, can upregulate atrial natriuretic peptide (ANP), a marker of cardiac myocyte hypertrophy (50).

Application of mechanical stimuli. The static strech device that we employed has also been previously described (20, 41). In this design, a silicone elastic membrane is secured within a membrane holder by an O-ring. The membrane holder is fixed on top of a mobile plate, and beneath the plate is a cylinder with a square Teflon indenter that produces a principal stretch oriented along both axes of the indenter. The plate is attached to a cam, which is rotated by a direct-current motor to generate a vertical sinusoidal displacement of the plate. This pushes the membrane against the indenter and thus deforms the silicone membrane. The use of a square indenter produces a biaxial stretch on this membrane. Stretch amplitude is controlled by varying the extent of displacement, and frequency is controlled by the direct-current motor speed.

Measure of IGF-I concentration. Conditioned media from stretched CFb and from nonstretched CFb were collected for IGF-I measurements. This culture medium (10 or 30 ml) was concentrated at 4°C using Centrifugal Filter Devices (Millipore, Billerica, MA), and the final volumes were recorded. IGF-I concentrations were determined by a sandwich ELISA that is designed to be specific for rat IGF-I (Diagnostic Systems, Webster, TX) (15, 40). The sensitivity of this ELISA assay is reported to be 30 ng/ml (as stated in the directions for use). In brief, microtiter strips coated with primary IGF-I antibody to capture IGF-I were incubated with samples for 1 h at room temperature (~25°C) while being gently agitated on a rotary shaker plate (500–700 rpm). Captured IGF-I was detected using hors eradish peroxidase-linked secondary anti-IGF-I antibody and visualized by tetramethylbenzidine. Standard curves and positive controls were performed for each assay, and IGF-I concentrations were obtained by interpolation. In addition, intra-assay (coefficient of variation = 4–6%) and interassay (coefficient of variation = 5–7%) analyses were performed.

Reagents. Thapsigargin (Sigma) was dissolved in DMSO to yield a stock solution having a concentration of 1.5 M. CFb viability after culture with thapsigargin at concentrations used in experiments was evaluated by the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay (38). Verapamil (Calbiochem, San Diego, CA) was dissolved in distilled water (4.9 mM stock solution) and used at a final concentration of 5 μM. Mouse IGF-I (Sigma) was reconstituted in phosphate-buffered saline containing BSA to yield a stock solution with a concentration of 50 μg/ml.

Statistical analysis. All results are expressed as means ± SD. Statistical evaluations were performed using ANOVA (with Bonferroni t-test post hoc testing) or Student’s t-test. Significance was accepted for data sets with P < 0.05.

Results

Static stretch does not increase secretion of IGF-I from CFb.

In our initial series of experiments, a static stretch device (23) was used to examine whether this mode of mechanical perturbation can modulate IGF-I secretion. CFb were seeded onto fibronectin-coated silicone membranes that were mounted in this stretch device while being incubated in 10% FBS-DMEM. After CFb attachment (24 h), the cells were refreshed with 0.1% FBS-DMEM for an additional 24 h. A biaxial strain of either 3%, 6%, or 10% was applied for a period of 24 h. The concentration of IGF-I released by CFb into the conditioned media was measured by ELISA. Samples were collected and assayed from nonstretched (control) and stretched CFb. As shown in Fig. 1B, the results consistently showed that static stretch did not alter IGF-I secretion: IGF-I concentrations in the cultured CFb were shown not to differ significantly from mean biaxial strains measured in the substrate deformation for nominal strains of 0–10%. Strains along circumferential and radial axes were equal in magnitude and homogeneously distributed with negligible shear (23). Before the experiments, each stretcher was calibrated for an applied stretch between 0–10%.

The cyclic-stretch device that we employed has also been previously described (20, 41). In this design, a silicone elastic membrane is secured within a membrane holder by an O-ring. The membrane holder is fixed on top of a mobile plate, and beneath the plate is a cylinder with a square Teflon indenter that produces a principal stretch oriented along both axes of the indenter. The plate is attached to a cam, which is rotated by a direct-current motor to generate a vertical sinusoidal displacement of the plate. This pushes the membrane against the indenter and thus deforms the silicone membrane. The use of a square indenter produces a biaxial stretch on this membrane. Stretch amplitude is controlled by varying the extent of displacement, and frequency is controlled by the direct-current motor speed.

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H678

IGF-I secretion from stretched ventricular fibroblasts

under nonstretched conditions (15). Accordingly, it has been hypothesized that adult CFb might also secrete IGF-I in this manner under nonstretched conditions. In contrast, it is also plausible that dynamic mechanical stimuli may induce increased expression, synthesis, and secretion of IGF-I.

The present experiments were conducted to determine whether IGF-I secretion is modulated by stretch in CFb. Both static and dynamic stretch systems were used to apply stretch to adult rat ventricular fibroblasts (CFb). The results revealed a role of cyclic mechanical force (biaxial strain) in regulating IGF-I secretion in CFb. Furthermore, we have demonstrated that IGF-I, at concentrations comparable with those that have been measured in mechanically stretched CFb, can upregulate atrial natriuretic peptide (ANP), a marker of cardiac myocyte hypertrophy (50).
the conditioned media obtained from stretched CFb were not significantly different from the values of the conditioned media in nonstretched CFb. This lack of effect on static stretch was not due to the particular time point selected for measurement. All time-course experiments, in which the CFb were subjected to 10% strain for either 6, 12, or 24 h, showed a very similar pattern of negative results (Fig. 1A). In combination, these results demonstrate that static stretch does not induce or modulate IGF-I secretion from CFb.

Cyclic stretch increases secretion of IGF-I from adult CFb in a frequency- and time-dependent manner. In our next set of experiments, a cyclic stretch device (20, 41) was used to determine whether this more physiological pattern of applied strain can modulate IGF-I secretion. CFb were subjected to 10% cyclic strain for either 6, 12, or 24 h at 1 Hz (Fig. 2A). In contrast to the static stretch data, a significant time-dependent increase in the release of IGF-I was observed. As shown in Fig. 2A, after 6 h of cyclic stretching, stretched cells showed no significant difference from nonstretched control values in IGF-I secretion. In contrast, at the 12-h time point, stretched CFb secreted a greater amount of IGF-I than nonstretched cells (2.25 ± 0.36 vs. 0.96 ± 0.09 pmol/1E6 cells, 2-fold increase, P < 0.05). At the 24-h time point, IGF-I secretion from cyclic-stretched cells resulted in a threefold increase over nonstretched control cells (5 ± 0.53 vs. 1.76 ± 0.3 pmol/1E6 cells, P < 0.01).
cells, $P < 0.01$). Reducing the frequency of stretching from 1 to 0.5 Hz did not alter this pattern of secretion of IGF-I; that is, as observed in the 1 Hz experiments, the IGF-I concentrations at 0.5 Hz (Fig. 2B) in the conditioned media at the first time point (6 h) were not different between stretched and nonstretched CFb. However, at 0.5 Hz, the secretion of IGF-I from stretched CFb at 12 h was significantly greater by twofold ($2.58 \pm 0.38$ vs. $1.05 \pm 0.2$ pmol/1E6 cells, $P < 0.05$) than that from nonstretched CFb. At this frequency, the 24-h time point data also showed a significant increase in IGF-I concentration by 3.5-fold ($3.34 \pm 0.95$ vs. $0.97 \pm 0.08$ pmol/1E6 cells, $P < 0.01$). Interestingly, a further reduction in the frequency of stretching (from 0.5 Hz to 0.1 Hz) resulted in the IGF-I not increasing above control values (Fig. 2C); that is, in response to a stretch frequency of 0.1 Hz, there was no increase in IGF-I secretion in stretched CFb compared with nonstretched CFb, even at 24 h. These results show that dynamic/cyclic stretch can significantly increase IGF-I secretion. This effect takes ~12 h to develop and is frequency dependent.

Cyclic stretch increases expression of IGF-I mRNA from adult CFb in a time-dependent manner. To determine whether the observed increases in IGF-I protein measured in the conditioned media were potentially due to increased synthesis of IGF-I, we performed quantitative real-time PCR on CFb that had been subjected to cyclic stretch. When IGF-I mRNA expression levels were normalized to the nonstretched controls, an approximate threefold increase was observed at the 24-h time point. This therefore demonstrates that cyclic stretch can modulate IGF-I expression at the transcriptional level (Fig. 3).

Changes in extracellular [Ca$^{2+}$] and block of L-type Ca$^{2+}$ channels do not affect IGF-I secretion by cyclic stretch. To determine whether the increased IGF-I release by cyclic stretch was dependent on extracellular Ca$^{2+}$ concentrations ([Ca$^{2+}$]$_o$), CFb were stretched at 1 Hz in media containing either 0.3 or 4 mM [Ca$^{2+}$]$_o$. These results were compared with those from control [Ca$^{2+}$]$_o$ amounts (1.8 mM, Fig. 4A). These results showed that the stretch-induced IGF-I release is not dependent on [Ca$^{2+}$]$_o$. CFb in 1.8, 0.3, or 4 mM [Ca$^{2+}$]$_o$ all exhibited significant IGF-I secretion (3-, 1.7-, and 2.1-fold, respectively, $P < 0.05$). However, these were not significantly different from each other.

To further evaluate the potential role of Ca$^{2+}$ influx on IGF-I secretion, CFb were stretched in the presence of verapamil (5 μM), an L-type Ca$^{2+}$ channel blocker. After 24 h of cyclic stretch, IGF-I secretion increased by 1.7-fold in the presence of verapamil and 3-fold in control samples without verapamil. Again, these differences were not statistically significant (Fig. 4B). These findings indicate that transmembrane influx of Ca$^{2+}$ from the extracellular medium through L-type Ca$^{2+}$ channels is not essential for the induction of IGF-I in response to stretch in these CFb.

Intracellular [Ca$^{2+}$] stores are involved in cyclic stretch-induced secretion of IGF-I from CFb. Thapsigargin is an inhibitor of the Ca$^{2+}$-ATPase or Ca$^{2+}$ pump that sequesters Ca$^{2+}$ back into intracellular Ca$^{2+}$ stores in mammalian cells. Thapsigargin, therefore, effectively inhibits reuptake of Ca$^{2+}$ that has been released from the endo- or sarcoplasmic reticulum. This results in increased cytosolic Ca$^{2+}$ and depletion of the intracellular stores (17, 33). In this study, CFb were treated with thapsigargin (50–100 nM) while being stretched at 1 Hz for 24 h. These results were compared with nonstretched CFb that received an identical thapsigargin treatment. As shown in

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**Fig. 4.** Effects of extracellular Ca$^{2+}$ on stretch-induced IGF-I release from CFb. A: medium with extracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$_o$) of 0.3, 1.8, or 4 mM was added immediately before cyclic stretch was applied. In all cases, the groups of CFb were stimulated by 10% stretch, at 1 Hz, and for 24 h. IGF-I release under each [Ca$^{2+}$]$_o$ condition was compared with nonstretched controls. Conditioned media were collected and assayed for IGF-I concentration by ELISA. Results are normalized to total cell number from each experimental condition. Data are presented as means ± SD ($n = 5$ experiments). B: 5 μM verapamil (5 μM), an L-type Ca$^{2+}$ channel blocker, was added immediately before cyclic stretch was applied (10%, 1 Hz, 24 h). Conditioned media were collected and assayed for IGF-I concentration by ELISA. Results are normalized to total cell number from each experimental condition. Data are presented as means ± SD ($n = 5$ experiments).

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**Fig. 3.** The dependence of IGF-I mRNA expression in CFb as a function of duration of cyclic stretch. Results are normalized to nonstretched controls from each experimental condition. Data are presented as means ± SD ($n = 5$ experiments). *$P < 0.05$ vs. 6 or 12 h.
physiological levels of Ca²⁺ by markedly increasing IGF-I secretion and upregulating first evidence that CFb respond to cyclic, but not static, stretch maintained in primary culture. These findings also provide the that adult ventricular fibroblasts (CFb) secrete IGF-I when paracrine manner.

DISCUSSION

Fig. 5, CFb treated with thapsigargin for 24 h during application of mechanical stretch failed to exhibit any significant stretch-induced secretion of IGF-I.

IGF-I upregulates ANP expression in cardiac myocytes. In the hypertrophic heart, IGF-I expression has been shown to increase (12, 18). ANP is a well-known marker of hypertrophy in myocytes (50). We investigated whether CFb can secrete IGF-I at concentrations sufficient to act as a paracrine factor on myocytes. This was assessed by measuring ANP mRNA expression in myocytes that were cultured in exogenous IGF-I at concentrations comparable with those measured from cyclically stretched CFb. We found that when myocytes were incubated with conditioned media from stretched CFb, the results were inconsistent (data not shown). This was suspected to be due to other paracrine factors that were also secreted by the CFb, based on multiple protein bands that were detected by SDS-PAGE. Therefore, we instead applied purified IGF-I to myocytes. IGF-I to myocytes at concentrations comparable with those measured in the conditioned media produced an upregulation of ANP mRNA expression (Fig. 6). This was found to be significant at 10⁻⁶ M (P < 0.05). This suggests that CFb may be capable of affecting myocyte responses in a paracrine manner.

Summary of main findings. Our results show for the first time that adult ventricular fibroblasts (CFb) secrete IGF-I when maintained in primary culture. These findings also provide the first evidence that CFb respond to cyclic, but not static, stretch by markedly increasing IGF-I secretion and upregulating mRNA expression. The effect on secretion is not dependent on physiological levels of [Ca²⁺]o and is not modulated by blocking L-type Ca²⁺ channels. However, it is dependent on a thapsigargin-sensitive pool of intracellular Ca²⁺.

IGF-I is a growth factor that mediates its effects through a number of autocrine/paracrine pathways. Major IGF-I effects include functioning as a stimulant for cell growth, proliferation, and survival. Quite recently, IGF-I has been referred to as a “cardiac hormone” by Ren et al. (36) when they reviewed the role of IGF-I in cardiovascular growth and function, as well as in the development of cardiac hypertrophy. Our results demonstrate that CFb can secrete IGF-I at concentrations sufficient to upregulate ANP, a known marker of cardiac hypertrophy in myocytes (50).

Relation to previous literature. Although it has been reported that cultured neonatal rat CFb can secrete IGF-I (15), the regulation of its release is not well understood, and no previous studies have addressed this in adult myocardium. Responses to applied or intrinsic mechanical forces have been studied in many cell types. Well-established stretch-mediated responses include gene expression regulation (39), cell proliferation (48), and peptide growth factor induction (25, 46). Using a static stretch system, van Wamel et al. (46) showed that stretch could cause neonatal CFb to release TGF-β1. Partly for this reason, in our initial experiments, a static strain was imposed on CFb in culture to determine whether specific components of applied strain can potentially regulate IGF-I secretion. Our results showed that static strain had no effect on the release of IGF-I from CFb.

A number of papers have shown that dynamic or cyclic stretch can significantly influence cell responses. For example, in cultured vascular smooth muscle cells, release of FGF-2 is enhanced as a function of the frequency of applied strain (7). To address whether CFb also may have the ability to sense and react to stretch frequency, we subjected CFb to cyclic biaxial strain. Our findings clearly showed that a release of IGF-I can be regulated by cyclic strain. Furthermore, there appears to be a frequency threshold. At a frequency below 0.5 Hz, cyclic strain did not cause a release of IGF-I. When strains exceeded the frequency threshold, IGF-I secretion from stretched CFb significantly increased. The nature of IGF-I release from CFb regulated by frequency of stretch implies that, under abnormal cardiac conditions (e.g., elevated heart rate or mechanical stress such as hypertension), CFb may respond by altering levels of IGF-I secretion. Physiological studies in mice have demonstrated that IGF-I can improve cardiovascular function measured in terms of cardiac output, stroke volume, left ventricular end systolic/diastolic pressures, ventricular dilation,
wall stress, and cardiac hypertrophy (24, 27). Additional studies with cultured myocytes have demonstrated that IGF-I can decrease myocyte apoptosis (47). In contrast, an overexpression of IGF-I has been implicated in myocyte hypertrophy (28, 31).

Our results also reveal that cyclic stretch duration is a determinant for augmented IGF-I secretion from mechanically stimulated CFb. In vascular smooth muscle cells, both acute (24 h) and chronic (6 day) cyclic stretch induced IGF-I secretion that increased with time (12). In skeletal muscle cells, repetitive stretch/relaxation stimulated acute release of IGF-I during the first 4 h but caused no additional increase in secretion over 24 h (13). We found that IGF-I release from stretched CFb increased with the duration of strain over a 24-h period. In summary, the available data suggest that IGF-I secretion is a transient or persistent response in different cell types and that its mechanotransduction is cell-type specific and may be associated with the unique functional demands of the cells in their tissue environment.

A number of ways in which mechanical stimulation can modulate excitation-secretion coupling have been suggested in mechanosensitive cells (4, 21, 42). One example is the regulated secretion of peptides and hormones. This mode of excitation-secretion coupling response depends on \([Ca^{2+}]_o\) and/or L-type \(Ca^{2+}\) channel expression/activation. Accordingly, we examined the role of \([Ca^{2+}]_o\) in mechanotransduction of IGF-I release. However, in CFb, either increasing or decreasing \([Ca^{2+}]_o\) in the physiological range did not modulate stretch-induced IGF-I secretion. Moreover, the L-type \(Ca^{2+}\) channel blocker verapamil had no effect on stretch-induced IGF-I secretion. Thus \(Ca^{2+}\) influx is not a primary trigger for stretch-induced IGF-I release in CFb from adult hearts.

In a variety of cell types, intracellular \(Ca^{2+}\) stores can strongly modulate secretion. For example, it has been shown that thapsigargin increases intracellular \(Ca^{2+}\) levels in adult rat CFb (33) and that thapsigargin significantly decreases IGF-I in conditioned media from skin fibroblasts of fetal rats after 48 h (17). The effect of thapsigargin on stretch-induced IGF-I secretion has not been studied. In the present study, thapsigargin almost completely inhibited the stretch-induced enhancement of IGF-I secretion from CFb. This finding indicates that the release of IGF-I mediated by stretch may be dependent on intracellular \([Ca^{2+}]\) or on a signaling mechanism that requires intact intracellular stores.

Our results demonstrate that long-term application of thapsigargin can block the synthesis or release of IGF-I from rat ventricular fibroblasts. Although the mechanism for this effect is not fully understood, and in fact is not addressed in a comprehensive way by the experimental results in this article, recent literature suggests one plausible hypothesis. In a number of mammalian cells, so-called capacitative \(Ca^{2+}\) entry is strongly modulated by the \(Ca^{2+}\) content in intracellular stores (35); in some cases the surface membrane conductance that is responsible for this flux is the transient receptor potential (TRP) channel family (9). We and others have demonstrated (using both electrophysiological and molecular techniques) that a number of different TRP channels are expressed in rat ventricular fibroblasts (2, 37). Activation of these TRP channels can result in an inward current carried by both \(Na^+\) and \(Ca^{2+}\) (34). There is some evidence to suggest that this flux can regulate \(Ca^{2+}\)-induced \(Ca^{2+}\) release from intracellular stores in what has been termed “a two-step intracellular release process” (32). Thapsigargin application effectively depletes the endoplasmic reticulum of \(Ca^{2+}\) and thus alters intracellular \(Ca^{2+}\) homeostasis. This may change TRP channel activity and inhibit intracellular \(Ca^{2+}\) release. In general, the magnitude and the time course of changes in intracellular \(Ca^{2+}\) are known to alter the synthesis and release of peptides and hormones within the cell in what has been termed the “AM and FM of calcium signaling” (3).

In conclusion, we observed that adult rat ventricular fibroblasts are a source of IGF-I. The stretch-induced secretion of IGF-I exhibits both frequency and time dependence. IGF-I plays numerous roles in both physiological and pathophysiological conditions. In studies with cultured myocytes, IGF-I was found to decrease myocyte apoptosis (47). It has also been shown to be effective in animal models of myocardial infarction and ischemia-reperfusion. The administration of IGF-I after injury minimized myocardial damage (5, 26). In contrast, an overexpression of IGF-I has been implicated in myocyte hypertrophy (28, 31). We have shown that mechanical forces can significantly alter secretion of IGF-I in CFb. Thus our findings provide new insights into how mechanotransduction may alter IGF-I levels which can maintain normal cardiac physiology or initiate pathological responses.

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