Stretch-induced VEGF Expression in the Heart

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Abstract

Vascular endothelial growth factor (VEGF) is an endothelial cell mitogen involved in vascular development and angiogenesis. Recently we have observed increased VEGF expression in the normal myocardium after myocardial infarction in a rat heart. This study was designed to explore the mechanism responsible for this increase in VEGF expression. Induction of myocardial stretch in an isolated perfused Langendorff preparation by inflation of an intraventricular balloon to an end-diastolic load of 35 mmHg for 30 min resulted in a nearly sixfold increase in VEGF message level not only in the chamber subjected to stretch (left ventricle) but also in the unstretched right ventricle, thus raising the possibility of a soluble factor mediating stretch-induced induction of VEGF expression. This was further confirmed by demonstrating that coronary venous effluent collected from the stretched heart and used to perfuse isolated hearts in which no balloon was present was able to induce VEGF expression in these normal hearts.

Inhibition of TGF-β activity using a neutralizing antibody, but not antagonists/inhibitors of endothelin and angiotensin II, eliminated stretch-induced increase in VEGF expression. Staurosporine, a protein kinase C inhibitor, also blocked stretch-induced increase of VEGF expression. Measurement of TGF-β concentration in the perfusate demonstrated increased amounts of the cytokine after myocardial stretch, and addition of TGF-β protein to the perfusion buffer resulted in increased VEGF expression in control hearts. These results suggest that stretch-induced increase of VEGF expression in the heart is mediated at least in part by TGF-β. (*J. Clin. Invest.* 1997. 100:18–24.) Key words: angiogenesis • growth factors • TGF-β • endothelial • shear stress

Introduction

Vascular endothelial growth factor (VEGF)[1] is an endothelial-specific cell mitogen intimately linked with new vessel development. During embryonic development VEGF is predominantly produced in tissue acquiring new capillary networks, and VEGF knockout is lethal at the heterozygote stage with mice showing abnormal blood island formation and vasculogenesis (1, 2). Similar, albeit less severe, defects are observed with homozygous knockout of VEGF receptors with flk-1 knockout mice demonstrating abnormal blood island development (3) and flt-1 knockout mice demonstrating abnormal vasculogenesis (4). In adult tissues, VEGF expression is significantly increased by hypoxia and/or tissue ischemia. In particular, myocardial ischemia is associated with a sevenfold increase in the growth factor's expression (5–7), and increased VEGF expression persists in chronically ischemic myocardium (8).

In recent studies of VEGF expression in a rat myocardial infarction model of angiogenesis (9), we observed increased expression in the areas of the left ventricle remote from the infarct area as well as in the right ventricular myocardium (the chamber not directly affected by left ventricular infarction). Since these areas appeared to be neither ischemic nor hypoxic, we postulated that a different stimulus was responsible for the observed induction of VEGF expression. One such plausible stimulus is myocardial stretch occurring secondary to a rising left ventricular end-diastolic pressure typically observed in hearts with extensive left ventricular infarctions. However, the infarct model does not provide an adequate opportunity to test this hypothesis since myocardial infarction and associated tissue necrosis lead to extensive infiltration of inflammatory cells secreting cytokines capable of stimulation of VEGF expression.

Therefore, to explore a possible link between a pure mechanical stretch and VEGF gene expression, in this study we used an isolated rat heart Langendorff model that allows easy manipulation of myocardial stretch by introduction of an inflatable left ventricular balloon. Using this model, we have been able to observe stretch-induced activation of VEGF expression and to explore mechanisms of this effect.

Methods

Isolated heart preparations

Langendorff heart preparation. Hearts excised from anesthetized, heparinized (500 U/100 g body wt), male Sprague-Dawley rats weighing between 350 and 400 g were immediately placed in a preweighed beaker with ice-cold buffer solution and the lungs and connective tissue were quickly trimmed. The aorta was connected to an 18 gauge stainless steel feeding needle through which oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit solution (mmol/liter: NaCl 118.0, KCl 4.7,

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1. *Abbreviations used in this paper:* CVE, coronary venous effluent; LV, left ventricle; PKC, protein kinase C; RV, right ventricle; VEGF, vascular endothelial growth factor.

KH_2PO_4 1.2, CaCl 1.0, $MgCl_2$ 1.2, $NaHCO_3$ 23.0, and dextrose 10.0, pH = 7.4) was pumped at a rate of 10 ml/min per gram of heart weight. The cannula was connected to a bubble trap immediately upstream of the aorta which was connected to a fourway stop cock with a side arm connected to a Statham P23b pressure transducer (Gould Inc., Cleveland, OH) to measure coronary perfusion pressure. An incision was made in the right ventricle (RV) at the junction with the pulmonary artery to create a drain for coronary venous effluent (CVE), and the heart was paced at 180 bpm.

A soft intraventricular balloon was passed through the mitral annulus until seated in the left ventricle (LV), and the distal end of the balloon assembly was connected to a pressure transducer. Thebesian venous return from the LV was emptied via a drain inserted in parallel with the balloon. The cannulated heart assembly was bathed in a beaker filled with the CVE which was drained at a rate commensurate with the flow rate (see Fig. 1 *A*). CVE was collected for later analysis or reoxygenated and used as a coronary perfusate (see below). All studies were carried out at 30°C.

Unstretched myocardium. The intraventricular balloon was inflated just enough to obtain a pressure signal to monitor preparation stability. After a 15-min stabilization period, the balloon was further inflated to achieve an end-diastolic pressure of $\sim$ 5 mmHg while maximizing the heart's capability of generating pressure and the collection of effluent was begun. After 30 min, perfusion was terminated and the heart was snap-frozen for further analysis.

Stretched myocardium. The intraventricular balloon was inflated just enough to obtain a pressure signal to monitor preparation stability. After a 15-min stabilization period, the balloon was further inflated to achieve a desired end-diastolic pressure (15, 25, or 35 mmHg) while maximizing the heart's capability of generating pressure and the collection of effluent was begun. After 30 min, perfusion was terminated and the CVE and the heart were snap-frozen for further analysis.

Aequorin loading. A small region of the stretched LV was injected with aequorin as previously described. Briefly, 3–5 μl of aequorin was injected with a glass micropipette into the interstitium of the epicardium of the inferoapical region of the LV, after which the entire isolated perfused heart preparation was placed in a light-tight box. The aequorin light signal was detected by photomultiplier from the apex of each heart and recorded as anodal current (10).

Tandem Langendorff preparation. As shown in Fig. 2 *A*, two isolated heart preparations were instrumented in series with the reoxygenated CVE from the first heart used to perfuse a second heart. The intraventricular balloon in the first heart was inflated just enough to record a pressure signal to monitor preparation stability and after a 15-min stabilization period it was further inflated to 5 (unstretched preparation) or 35 (stretched preparation) mmHg. For 30 min, the CVE from this preparation was then used to perfuse the second heart in which a piece of PE90 tubing, positioned in the LV and connected to a pressure transducer, was used to monitor the functional stability of the second heart. At the end of that time period, both hearts were snap frozen for further processing. In additional experiments, after 30 min of perfusion of the second heart with unstretched CVE, a new (third) heart prepared as described above was used to replace the second heart in the tandem preparation. The balloon in the primary heart was then inflated to 35 mmHg and the tandem heart was perfused for an additional 30 min.

Signal recording. LV isovolumic pressure, aequorin light signals, and coronary perfusion pressure were simultaneously recorded on a four channel strip-recorder (model 35-V704-10; Gould Inc.), and on a digital oscilloscope (model 4094A; Nicolet Instruments Corp., Madison, WI). Digitized LV pressure and aequorin recordings were stored on a disk recorder (model XF-44; Nicolet Instrument Corp.) connected to the oscilloscope. Customized software read data from the disk to calculate pressure, intracellular calcium, and associated functional variations.

Lactate production. Lactate measurements in the coronary effluent were carried out in a conventional manner (11) using an enzymatic assay (Lactate Reagent; Sigma Chemical Co., St. Louis, MO) by repeatedly sampling coronary effluent during and after intraventricular balloon inflation. Transient induction of ischemia was used as a positive control for the assay.

RNA analysis

Total RNA was obtained from stretched, perfused, and inhibitor-treated hearts by 4 M guanidinium isothiocyanate extraction followed by centrifugation through 5.7 M cesium chloride. Initially, the LV was carefully separated from RV then snap-frozen in liquid nitrogen. After myocardium was homogenized and centrifuged, the RNA pellet was dissolved in water, and then precipitated in ethanol. For Northern blot analysis, 10 μg RNA was fractionated on 1.3% formaldehyde agarose gel and transferred to GeneScreen Plus filter (DuPont, Wilmington, DE). The VEGF, TGF-β, and IL-1β cDNA probes were labeled with [α^{32}P]dCTP (New England Nuclear, Boston, MA) by a random-priming labeling kit (Boehringer Mannheim, Indianapolis, IN) and purified of unincorporated nucleotides using G-50 Quick Spin Columns (Boehringer Mannheim). The typical specific activity of the probes used in the experiments was 1–2 $\times$ 10^9 cpm/μg. The blots were hybridized at 68°C for 3 h in QuikHyb solutions (Stratagene Inc., La Jolla, CA). After the hybridization, blots were washed twice in 2 $\times$ SSC, 0.1% SDS for 15 min at room temperature and then twice in 0.1 $\times$ SSC, 0.1% SDS for 15 min at 60°C. Autoradiography was carried out with Kodak XAR film (Eastman Kodak Co., Rochester, NY) at 80°C for 16–20 h. For quantitative analysis of expression, the blots were exposed on PhosphorImager (Molecular Dynamics, Sunnyvale, CA) and analyzed using ImageQuant software (Molecular Dynamics).

Immunoblotting

Total protein from myocardial tissues was obtained by homogenizing in a lysis buffer containing 1% NP-40, 0.5% sodium deoxycholate, and 0.1% SDS, centrifuging at 3,000 *g* for 10 min. The effluent collected from isolated perfused stretched or unstretched hearts was concentrated using Centriprep 3 (Amicon Inc., Beverly, MA) columns to a final volume of 1 ml. Protein content of the supernatant was determined using Bradford as modified for the DC protein assay (Bio-Rad, Hercules, CA). 20 mg of total protein from each buffer preparation (stretched or unstretched hearts) was fractionated on 10% SDS-polyacrylamide gel transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore, Bedford, MA). The membrane was incubated with 5% skim milk in Tris-buffered saline for 30 min to block nonspecific absorption. TGF-β was subsequently detected by incubating the membrane with a 1:1,000 dilution of polyclonal anti–human TGF-β1 antibody (Santa Cruz Biotech, Santa Cruz, CA) for 2 h at room temperature followed by incubation with a 1:1,000 dilution of horseradish peroxidase–conjugated goat anti–rabbit IgG for 1 h. All Western blots were quantified using densitometry.

All experiments were performed in triplicate and the data are presented as mean±standard deviation.

Results

Stretch induction of VEGF expression. To assess the effect of myocardial stretch on VEGF mRNA levels in the normal rat heart, a soft balloon was placed in the LV and inflated to 35 mmHg of pressure (Fig. 1 *A*). Recording of LV pressure and aequorin light signal demonstrated stable contractile and intracellular calcium characteristics during 30 min of stretch (Fig. 1 *B*). At the end of that time, the heart was harvested, and total RNA was separately extracted from the LV and RV. Northern analysis of expression (Fig. 1 *C*) showed a 5.5-fold increase in VEGF mRNA levels in the LV and a nearly fourfold increase in the unstretched RV. Western analysis showed a comparable increase in VEGF protein (data not shown). Insertion of an

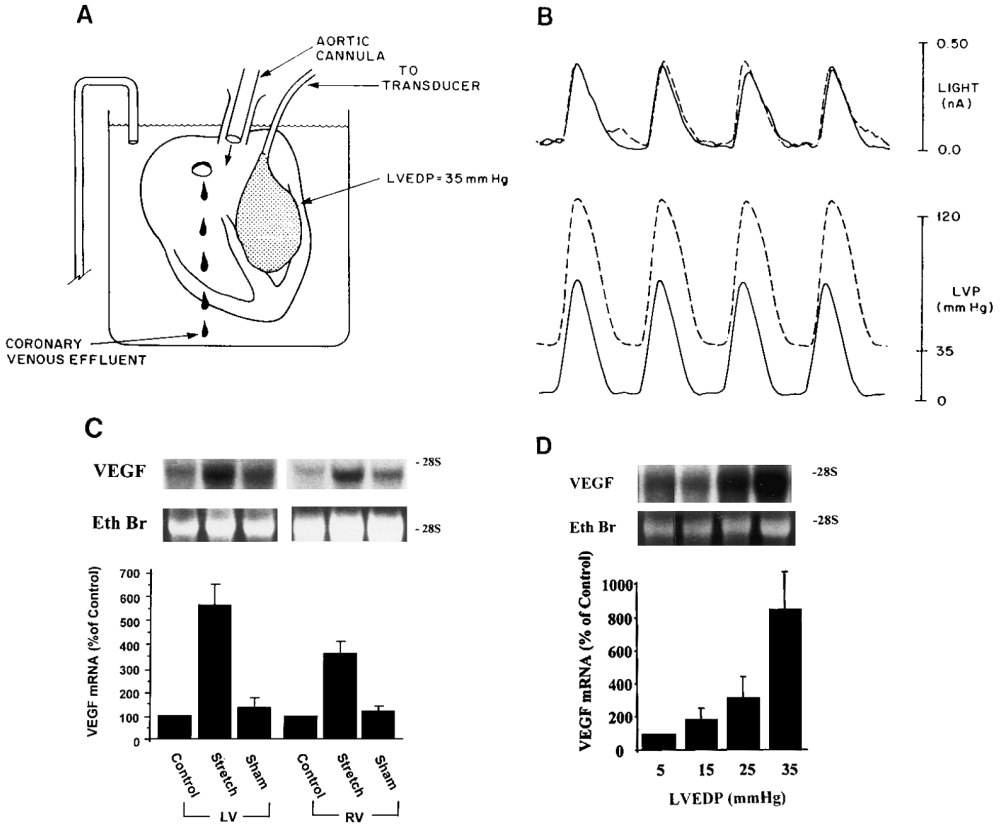

Figure 1. VEGF expression in an isolated heart preparation. Preparation of an isolated rat heart Langendorff model performed as described in the Methods section was followed by an insertion of an inflatable LV balloon (*A*). After aequorin loading, light signal (*B*, *top panel*) and LV pressure signal (*LVP*, *B*, *bottom panel*) were recorded during balloon inflation to 5 mmHg (*solid lines*) and 35 mmHg (*dashed lines*). Note indistinguishable light aequorin light signal in stretched and unstretched hearts suggesting the absence of ventricular ischemia. Northern analysis of VEGF expression (*C*) demonstrates increased cytokine mRNA expression after 30 min of stretch in both LV and RV. VEGF mRNA levels by RNA blot (*C*, *top bar*) were adjusted for RNA loading (ethidium bromide [*Eth Br*]) and averaged for three independent experiments (bar graph, mean±SD). The effect of different LV end-diastolic pressures (*LVEDP*) was evaluated by inflating an LV balloon to 15, 25, and 35 mmHg. Northern analysis demonstrates load-dependent increase in VEGF LV mRNA levels after 30 min of balloon inflation (*D*, *top*). Quantitative RNA analysis (a mean of two experiments) is shown on the bottom.

uninflated balloon (5 mmHg pressure) or simple maintenance of the heart on the perfusion apparatus for a comparable length of time did not alter VEGF message levels.

Since myocardial ischemia is known to result in increased VEGF expression, we conducted a series of studies to determine whether in our in vitro model initiation and maintenance of balloon inflation may have resulted in induction of global or subendocardial myocardial ischemia. No change in the epicardial aequorin signal was detected during or after 30 min of stretch at 35 mmHg end-diastolic pressure (Fig. 1 *C*), thus suggesting that no global ischemia was present. Evaluation of various hemodynamic parameters demonstrated no significant changes before or after 30 min 30 mmHg balloon expansion in peak systolic pressure (72±3 vs. 70±8 mmHg), end-diastolic pressure (8±1 vs. 3±3 mmHg), developed pressure (63±3 vs. 67±8 mmHg), positive dP/dt (1,643±159 vs. 1,872±72 mmHg/s), negative dP/dt (1,231±88 vs. 1,374±71 mmHg/ms), or time for 90% relaxation (1,473 vs. 135±9 ms). Furthermore, no lactate production was found in the sample buffer during or after 30 min of stretch.

To further evaluate the relationship between the magnitude of stretch and the degree of induction of VEGF expression, we evaluated myocardial VEGF mRNA after 30 min of stretch with an LV balloon inflated to 15 and 25 mmHg. Northern analysis demonstrated progressively increasing VEGF levels with increasing severity of stretch (Fig. 1 *D*).

The observed increase in VEGF expression could have occurred secondary to a direct stretch-induced signaling resulting in increased VEGF expression or may have been mediated by a stretch-induced release of a soluble substance. To test the latter possibility a second heart was added in tandem to the first with the aortic buffer inflow coming from the outflow of the first heart. An uninflated balloon was then positioned in the LV of the first heart and the preparation was allowed to stabilize for 5 min (Fig. 2 *A*). At the end of that time, the LV balloon in the first heart was inflated to 35 mmHg of pressure for 30 min. RNA analysis of the LV myocardium demonstrated increased VEGF message levels in both the stretched heart as well as in the second perfused, unstretched heart (Fig. 2 *B*). Simply perfusing the second heart without stretching the first heart did not produce any change in VEGF expression. Thus, a diffusible factor secreted by the stretched myocardium appeared responsible for induction of VEGF expression.

Identification of VEGF-inducing activity. To define the nature of diffusible activity responsible for the induction of VEGF expression after myocardial stretch, a series of experiments was carried out using single and tandem heart systems. To determine the stability of the VEGF-inducing activity, perfusate from the stretched heart was collected and stored for 1 h at 4°C and then used to perfuse a normal unstretched heart. RNA analysis demonstrated a degree of induction of VEGF expression similar to that seen with immediate perfusion (Fig. 3, compare the second and third bars). Boiling of the collected perfusate from the stretched heart preparation (100°C, 10 min)

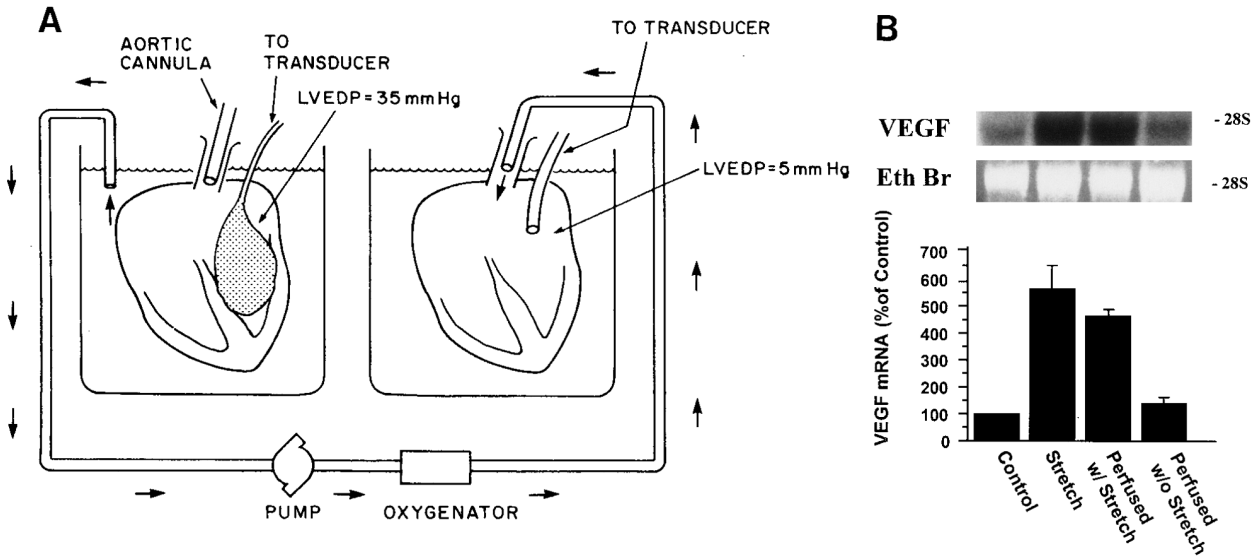

Figure 2. Analysis of VEGF expression in a tandem Langendorff preparation. Tandem preparation of two isolated rat hearts was accomplished as described in Methods with reoxygenated CVE from the first heart serving as coronary perfusate for the second heart (*A*). Inflation of an LV balloon to an LV end-diastolic pressure (*LVEDP*) of 35 mmHg in the first heart was associated with an increase in VEGF mRNA levels in the tandemly perfused heart (*B*, *Perfused w/ Stretch*) which was similar in magnitude to an increase in VEGF mRNA levels in the stretched (first) heart itself (*B*, *Stretch*). Note that perfusion without balloon stretch of the first heart did not affect VEGF mRNA levels. VEGF mRNA levels by RNA blot (*B*, *top bar*) were adjusted for RNA loading (ethidium bromide [*Eth Br*]) and averaged for three independent experiments (bar graph, mean±SD).

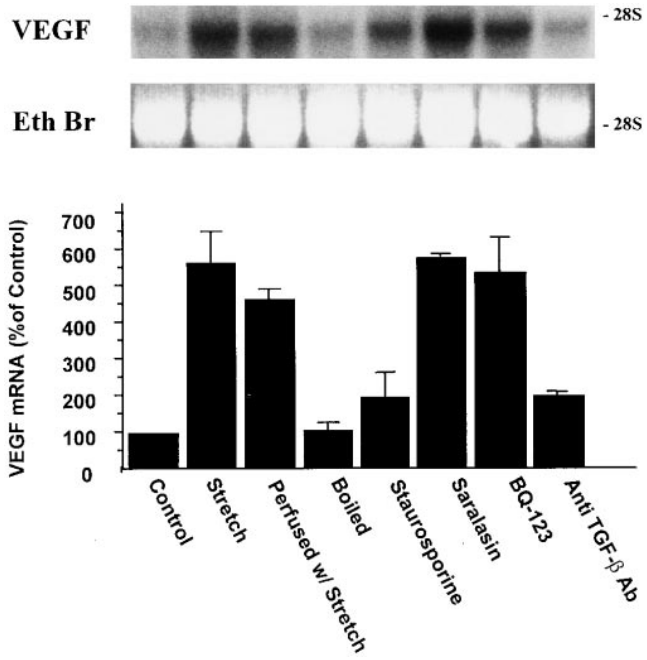

Figure 3. Regulation of stretch-induced activation of VEGF expression. Northern analysis of VEGF mRNA levels in the normal (control) heart (first lane), stretched heart (second lane), or unstretched heart perfused with an effluent from the stretched (35 mmHg) Langendorff preparation (third lane). Boiling (10 min, 100°C) of the CVE from the stretched heart but not the addition of saralasin or BQ-123 resulted in inhibition of stretch-induced VEGF expression. However, addition of PKC inhibitor staurosporine or a TGF-β neutralizing antibody inhibited activation of VEGF expression.

resulted in complete inactivation of VEGF-inducing activity (Fig. 3).

Since stretched myocardium is known to release several peptides including angiotensin II and endothelin-1, appropriate receptor antagonists (saralasin, 10^{-6} M, and BQ-123, 10^{-5} M) were added to the perfusion buffer. However, myocardial stretch with an inflatable balloon still resulted in activation of VEGF expression in the perfused unstretched heart. On the other hand, addition of protein kinase C (PKC) inhibitor staurosporine (7 nM) to the perfusion buffer resulted in a significant reduction of activation of VEGF expression, suggesting that the PKC pathway was involved in stretch-mediated activation of VEGF expression.

TGF-β as a mediator of stretch-induced activation of VEGF expression. TGF-β, a cytokine known to be present in the normal myocardium, can be released in response to mechanical stretch, is known to activate the PKC pathway, and can induce VEGF expression in vitro. To test whether the cytokine was responsible for induction of VEGF expression in this system, perfusate from a balloon-stretched heart was collected and divided into two equal fractions, one of which was incubated with a neutralizing anti–TGF-β (5 μg/ml) antibody while the other was incubated with an antibody buffer. After incubation, perfusion of a normal unstretched heart with a buffer-treated perfusate fraction demonstrated a previously seen increase in VEGF mRNA levels, while perfusion with anti–TGF-β antibody neutralized fraction demonstrated a marked reduction in induction of VEGF expression (Fig. 3).

The ability of TGF-β to induce VEGF expression in this system was demonstrated by adding the cytokine to the perfusion buffer of a control (unstretched) heart. RNA analysis

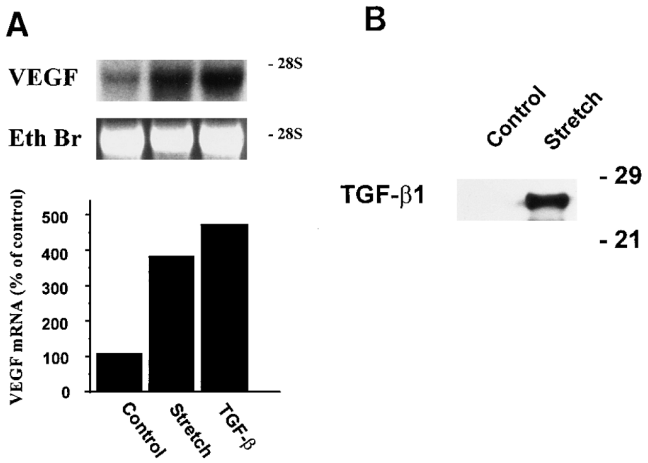

Figure 4. TGF-β and induction of VEGF expression. The ability of TGF-β to induce myocardial VEGF expression was tested in a rat Langendorff model. Addition of the cytokine (1 ng/ml) to an unstretched heart resulted in an increase of VEGF mRNA (*A*, TGF-β, third lane) similar to that seen with LV balloon inflation to 35 mmHg (*A*, *Stretch*). Immunoprecipitation using anti–TGF-β antibody resulted in detection of the cytokine in the CVE from the stretched but not control Langendorff rat heart preparation (*B*).

demonstrated a degree of increase in VEGF message levels similar to that seen with a stretched balloon-induced activation (Fig. 4 *A*). To show that myocardial stretch leads to increased TGF-β release, immunoprecipitation studies of buffers from stretched and unstretched hearts were carried out with anti-TGF-β antibody. Results show the presence of TGF-β protein in the buffer derived from the stretched heart but not from the unstretched heart (Fig. 4 *B*). At the same time, RNA analysis showed no change in myocardial TGF-β RNA levels (not shown).

Discussion

VEGF plays a key role in blood vessel formation during development as well as in angiogenesis taking place under a variety of normal and pathological conditions in mature tissues (12, 13). In addition, the growth factor is involved in regulation of vascular permeability, an activity that may play an important role in a number of processes (14). Increased VEGF expression is frequently observed in ischemic tissues including ischemic myocardium (7, 9, 15) as well as in a variety of tumors and other conditions associated with increased vascularity (16–19).

VEGF expression (both at the transcriptional and translational levels) is regulated in response to hypoxia (20–22) as well as by several cytokines including TGF-β (23, 24), IL-1β (25), and IL-6 (26), and is on all occasions associated with a physiologic (hypoxia, ischemia, wound healing) or pathologic (tumor growth, diabetic retinopathy) need for angiogenesis. However, to date there is no reported association between a mechanical stimulus such as myocardial stretch and upregulation of VEGF expression. Increases in the left ventricular end-diastolic pressure of the magnitude similar to that used in our study can occur under a number of conditions including myocardial infarction, congestive heart failure, long-standing hypertension, as well as several forms of valvular heart disease. Indeed, our observation of increased VEGF expression in the normal regions of the heart immediately after myocardial infarction (9) has prompted this study.

In this report we used an inflatable balloon catheter to achieve an increase in the left ventricle end-diastolic pressure similar to that seen with extensive myocardial infarction. This avoids the complications of the infarct model associated with ischemic cell injury and an influx of inflammatory cells secreting cytokines which can activate VEGF expression. Continuous recordings of left ventricular pressure and the rate of pressure rise (dP/dt, an index of myocardial contractility) generated by the beating heart with an inflated balloon remained stable throughout the study, suggesting that balloon inflation per se did not interfere with myocardial function. The onset of hypoxia or ischemia is associated with a quantitative and qualitative change in intracellular calcium which is reflected in aequorin light signal. Since the aequorin signal was unaffected by increase in mechanical stretch used in these studies (Fig. 1 *B*) it may be concluded that balloon inflation did not result in appreciable global myocardial ischemia (10). The occurrence of subendocardial ischemia is harder to exclude. However, we have not observed any deterioration in systolic or diastolic left ventricular function parameters during and after a balloon inflation to 35 mmHg and did not detect any lactate production during these experiments.

Using this isolated heart preparation we observed a significant and fairly prompt (within 30 min) rise in VEGF expression both in the chamber undergoing mechanical stretch (LV) as well as in a chamber (RV) not directly subjected to mechanical stretch. Further studies involving a tandem perfused heart preparation demonstrated that induction of VEGF expression was due to the presence of a soluble protein, while further ruling out balloon-induced subendocardial ischemia as a cause of increased VEGF expression.

Although in this study we have not determined cell types responsible for increased VEGF message levels, our previous study in a myocardial infarct model demonstrated that myocytes and invading macrophages were the two principal cell types responsible for VEGF synthesis (9). Given the absence of blood perfusion in the isolated heart model used in this study, myocytes remain the cell type most likely responsible for the observed increase in VEGF expression.

Several lines of evidence suggest that TGF-β is the factor responsible for induction of VEGF expression in this system. First, this cytokine has been shown previously to induce VEGF expression in cell culture (23, 24). In line with that observation, we demonstrated that the addition of TGF-β protein to the perfusion buffer induced VEGF expression in our isolated heart preparation. Secondly, TGF-β protein was detected in the effluent from the stretched heart but not from the unstretched heart. Finally, treatment of the perfusion buffer with a TGF-β neutralizing antibody eliminated VEGF-inducing activity. Activation of VEGF expression in this system appeared to depend on a PKC signaling pathway since the addition of staurosporine at low concentrations (presumed to affect PKC activity only) blocked stretch-induced activation of VEGF expression. This observation is in line with previous studies which have suggested PKC involvement in control of VEGF expression (27, 28) and the known ability of TGF-β to induce PKC activation (29). Several isoforms of TGF-β are known to occur in the heart. In addition to using a pan–TGF-β

neutralizing antibody, we used a TGF-β1 isoform specific antibody for detection of active protein in the coronary effluent, suggesting that this isoform is responsible for the observed stimulation of VEGF expression.

Demonstration of increased VEGF mRNA levels in this study could have occurred as a result of transcriptional activation of VEGF gene or, alternatively, due to increased VEGF message half-life. Several investigators have demonstrated that increases in VEGF expression seen with hypoxic stimuli are due to a combination of these effects (21, 22, 30). While TGF-β expression is known to be activated by shear stress (31) and mechanical pressure overload (32), our data are more consistent with stretch-induced release of TGF-β stored in the myocardium rather than stretch-induced activation of TGF-β transcription given the time course of relatively rapid increase in VEGF message levels (seen as early as 30 min after stretch induction) and the appearance of TGF-β protein in the effluent. It should be noted that while we detected TGF-β1 isoform using Western blotting, this method does not distinguish the active versus inactive form of the cytokine. An assay specifically designed to measure the active fraction (such as mink-lung bio-assay) may be of interest in further studies of TGF-β–VEGF interaction in this system.

It is interesting to speculate regarding the physiologic significance of this novel form of regulation of VEGF expression. TGF-β expression is known to be increased in myocardial hypertrophy, a condition which in its severe forms is associated with relative lack of adequate perfusion and easily inducible subendocardial ischemia. Thus induction of VEGF may serve as a signal for physiologically necessary compensatory angiogenesis which may ameliorate the effects of hypertrophy. Alternatively, rapid induction in VEGF expression after a rapid increase in left ventricular end-diastolic pressure (for example, in acute congestive heart failure) may serve to increase permeability of myocardial tissues, thereby increasing its turgor by raising water content. This increase in myocardial stiffness may then serve to counteract rising intracavitary pressure.

In summary, this study demonstrated that myocardial stretch is associated with rapid induction of VEGF gene expression which is mediated by TGF-β release from the stretched heart. The physiologic significance of this observation will require further investigations.

Acknowledgments

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