

# Peptide Growth Factors Can Provoke "Fetal" Contractile Protein Gene Expression in Rat Cardiac Myocytes

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## Abstract

Cardiac-specific gene expression is intricately regulated in response to developmental, hormonal, and hemodynamic stimuli. To test whether cardiac muscle might be a target for regulation by peptide growth factors, the effect of three growth factors on the actin and myosin gene families was investigated by Northern blot analysis in cultured neonatal rat cardiac myocytes. Transforming growth factor- $\beta$ 1 (TGF $\beta$ 1, 1 ng/ml) and basic fibroblast growth factor (FGF, 25 ng/ml) elicited changes corresponding to those induced by hemodynamic load. The "fetal"  $\beta$ -myosin heavy chain (MHC) was up-regulated about fourfold, whereas the "adult"  $\alpha$ MHC was inhibited > 50–60%; expression of  $\alpha$ -skeletal actin increased approximately twofold, with little or no change in  $\alpha$ -cardiac actin. Thus, peptide growth factors alter the program of differentiated gene expression in cardiac myocytes, and are sufficient to provoke fetal contractile protein gene expression, characteristic of pressure-overload hypertrophy. Acidic FGF (25 ng/ml) produced seven- to eightfold reciprocal changes in MHC expression but, unlike either TGF- $\beta$ 1 or basic FGF, inhibited both striated  $\alpha$ -actin genes by 70–90%. Expression of vascular smooth muscle  $\alpha$ -actin, the earliest  $\alpha$ -actin induced during cardiac myogenesis, was increased by all three growth factors. Thus, three  $\alpha$ -actin genes demonstrate distinct responses to acidic vs. basic FGF. (*J. Clin. Invest.* 1990. 85:507–514.) actin • myosin • fibroblast growth factor • transforming growth factor- $\beta$ 1 • cardiac hypertrophy

## Introduction

Cardiac hypertrophy provoked by a hemodynamic load comprises not only a quantitative increase in overall organ mass, individual myocyte volume, protein content and total RNA transcription, but also characteristic qualitative changes, including the expression of diverse sarcomeric, cytosolic, and membrane proteins as their embryonic isoforms (1). Up-regulation of the "fetal" contractile proteins,  $\beta$ -myosin heavy chain ( $\beta$ MHC<sup>1</sup>; 2, 3) and  $\alpha$ -skeletal actin ( $\alpha$ SkA; 4, 5), are perhaps

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1. Abbreviations used in this paper: aFGF and bFGF, acidic and basic fibroblast growth factor;  $\alpha$ CaA,  $\alpha$ -cardiac actin;  $\alpha$ MHC and  $\beta$ MHC,  $\alpha$ -

the most intensively studied of this ensemble. In principle, the transition from compensatory hypertrophy to intractable failure may in part be due to anomalous transcription of genes that encode proteins essential for cardiac function. However, while  $\beta$ MHC are thought to diminish myocardial contractility, on the basis of slower cross-bridge cycling (6), the possible physiologic implications of altered actin expression have not been proven. Thus, the changes produced by hemodynamic stress may recapitulate a fetal program whose elements share regulatory events, rather than adaptation, in common (5, 7).

Little is known of the specific transduction pathways through which pressure overload can coordinately regulate an ensemble of "embryonic" or "fetal" genes. Mechanical stimulation including passive stretch (8), or hormones induced by aortic constriction (9), may themselves induce hypertrophy or alter gene expression. There is increasing evidence that such signals might be coupled to cardiac mass through oncogene-encoded nuclear proteins such as *c-fos* and *c-myc* (5, 7, 10, 11), which have been implicated in the transduction of signals triggered by peptide growth factors (reviewed in references 12, 13). A potential role for peptide growth factors in cardiac hypertrophy, including transforming growth factor- $\beta$ 1 (TGF $\beta$ 1; 14) and the heparin-binding acidic and basic fibroblast growth factors (aFGF, bFGF; 15), has been suggested by their presence in developing and adult cardiac myocytes or the extracellular matrix. Furthermore, growth factor production increases in myocytes surviving coronary artery ligation (16), and autocrine or paracrine factors which accumulate in the myocardium during pressure-overload hypertrophy can stimulate cardiac growth in vitro (17).

That cardiac myocytes might be targets for the action of peptide growth factors also is suggested by the responses of skeletal muscle cells. Both bFGF and aFGF are potent mitogens for skeletal myoblasts (18) and block the onset of the myogenic phenotype in undifferentiated cells, apparently differing only in potency (18, 19). In contrast, TGF $\beta$ 1 suppresses the induction of muscle-specific proteins including MHC and  $\alpha$ -actin (20–23) in the absence of proliferative growth (21, 22). These effects may occur at least in part by preventing the appearance or activity of certain muscle-specific DNA-binding proteins which modulate transcription (24). Conversely, TGF $\beta$ 1 (21) and bFGF (25) also can down-regulate the muscle phenotype in myocytes which have not undergone terminal (irreversible) differentiation, whereas myocytes which are committed to fusion and the postmitotic state are reported to be refractory to the action of TGF $\beta$ 1 (20, 21) and bFGF (18) on muscle-specific genes.

However, it remains conjectural whether cellular events involved in growth factor signal transduction in fact play a role in cardiac hypertrophy triggered by pressure overload. First,

and  $\beta$ -myosin heavy chain;  $\alpha$ SkA,  $\alpha$ -skeletal actin;  $\alpha$ Sma,  $\alpha$ -vascular smooth muscle actin; TGF $\beta$ 1, type  $\beta$ -1 transforming growth factor.

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the specific actions of serum growth factors are contingent both on cell type (26) and developmental state (27). Extensive disparities distinguish cardiac development from that of skeletal muscle and other existing model systems, of which the most noteworthy, perhaps, are the ability to synthesize cardiac-specific proteins without exiting the cell cycle (28), the uncoupling of DNA synthesis from mitotic division shortly after birth (29), and, eventually, adaptive growth by cell enlargement (30). At least one oncogene which extinguishes the ability of skeletal muscle to differentiate, SV40 large T antigen (31), by contrast is permissive for a differentiated phenotype in cardiac myocytes (32). Moreover, recent reports demonstrate that commitment of pluripotent cells to the skeletal muscle pathway during embryogenesis may be conferred by a hierarchy of myogenic "determination" genes (33), most of which show homology to the nuclear oncogene *c-myc*, including MyoD1 (34) and myogenin (35, 36). In contrast, MyoD1 and myogenin are not expressed in cardiac muscle (34, 36), and the molecular mechanisms underlying cardiac ontogeny are not yet known. Together, these intrinsic differences suggest the likelihood that even genes which are co-expressed both by cardiac and skeletal myocytes must be subject to regulatory events which are lineage-specific. Genes such as  $\alpha$ -cardiac actin ( $\alpha$ CaA) and  $\alpha$ -SkA, which are targets for the action of peptide growth factors, might thus be expected to possess responses to growth factor binding that differ fundamentally in the environment of cardiac vs. skeletal muscle cells. Finally, significant differences are known to exist among contractile protein gene families in their response to particular trophic signals. For example, unlike the MHC genes,  $\alpha$ -CaA and  $\alpha$ -SkA are relatively insensitive to fluctuations in thyroid hormone concentration (37).

Accordingly, the specific objectives of the present investigation were to: (a) determine if cardiac myocytes in culture are targets for the suppressive effects of peptide growth factors on adult contractile protein gene expression; (b) establish if peptide growth factors can, in addition, elicit "fetal" contractile protein gene expression ( $\alpha$ SkA,  $\beta$ MHC, or both), as seen with pressure-overload hypertrophy; and (c) ascertain whether alterations in contractile protein gene expression necessarily are accompanied by increased total RNA, protein content, or cell number in myocardial cell cultures.

## Methods

**Cell culture.** Primary cultures of cardiac myocytes were prepared from the ventricles of 2-d-old Sprague-Dawley rats by enzymatic dissociation in 0.1% trypsin, 0.1% collagenase, and 0.025% DNAase (Worthington Biochemical Corp., Freehold, NJ). Cells were pooled in medium (Dulbecco's modified Eagle's medium/Ham's nutrient mixture F12 [1:1; Gibco Laboratories, Grand Island, NY], adjusted to 17 mM NaHCO<sub>3</sub>, 2 mM L-glutamine, and 50  $\mu$ g/ml gentamicin) supplemented with 10% fetal bovine serum (Hyclone Laboratories, Logan, UT). The cell population was partially depleted of mesenchymal cells by differential adhesiveness. Nonadherent cells were plated at 10<sup>5</sup> cells/cm<sup>2</sup> on 100-mm polystyrene dishes coated with 0.1% gelatin (ICN Biochemicals, Irvine, CA). After 24 h, the cells were washed and subjected to serum withdrawal for 48 h in serum-free medium supplemented with 1  $\mu$ g/ml insulin, 5  $\mu$ g/ml transferrin, 1 nM LiCl, 1 nM Na<sub>2</sub>SeO<sub>4</sub>, 25  $\mu$ g/ml ascorbic acid, and 1.0 nM thyroxine. The thyroid hormone concentration and cell density were higher than in a previous study (27) to ensure a more physiologic concentration and rate of contraction, respectively. Thereafter, cells were incubated for 24 h in

serum-free medium plus vehicle (control) or supplemented with TGF $\beta$ 1 (1 ng/ml), bFGF (25 ng/ml), or aFGF (25 ng/ml). The final concentrations for components of the vehicle applied to cultured myocytes were 4  $\mu$ M HCl, 10<sup>-7</sup> vol/vol Triton X-100 and 1  $\mu$ g/ml bovine serum albumin.

Myocyte-depleted mesenchymal cultures prepared from the rapidly adherent (30 min) fraction of ventricular cells were maintained for 5–7 d in F12 supplemented with 10% fetal bovine serum, passaged once at a 1:20 dilution, and subjected at confluency to mitogen withdrawal in the same serum-free medium utilized for the cardiac myocytes. Secondary cultures were utilized to overcome the limitation of residual myocytes found in primary mesenchymal cultures.

Acidic and basic FGF (R&D Systems, Minneapolis, MN) each were purified from bovine brain by three cycles of Heparin-Sepharose affinity chromatography, followed by immunoabsorption of the irrelevant peptide, and were at least 96% homogeneous by amino acid analysis. TGF $\beta$ 1 was used as the homodimer isolated from porcine platelets (R&D Systems).

**RNA isolation and Northern blot hybridization.** Total cellular RNA was isolated by the guanidinium thiocyanate-phenol-chloroform method (38) and quantitated by spectrophotometry. To minimize variance in the yield of RNA, each sample was pooled from four independent cultures. Yield was consistent between experiments (for four control cultures, the standard error of the mean was ~ 6% of the observed value). Aliquots (15  $\mu$ g per lane) were size-fractionated by formaldehydeagarose gel electrophoresis, and transferred to nylon membranes. To investigate actin and MHC gene expression, synthetic oligonucleotides were prepared to isoform-specific 3' untranslated sequences, as described previously (27, 37).  $\alpha$ -Smooth actin ( $\alpha$ Sma) mRNA was identified using a synthetic probe corresponding to 3' untranslated nucleotides 1195–1216 (39). Probes were labeled at the 5' end with [ $\gamma$ -<sup>32</sup>P]ATP using T4 polynucleotide kinase, to a specific activity of 4–6  $\times$  10<sup>8</sup> cpm/ $\mu$ g. Blots were washed for 45 min in 6 $\times$  SSC at room temperature and 20 min in 6 $\times$  SSC/1% SDS at 42°C. Blots were exposed to XAR-2 film (Eastman Kodak Co., Rochester, NY) at 70°C with intensifying screens and were quantitated by scanning densitometry.

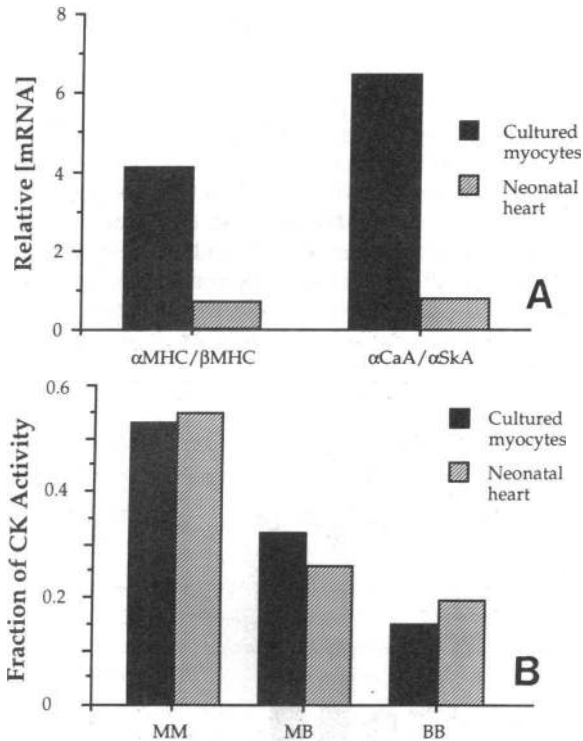
**Protein and creatine kinase determinations.** Cell pellets were disrupted using a Potter-Elvehjem homogenizer, in 0.32 M sucrose, 10 mM Tris-HCl (pH 8.0), and 5 mM  $\beta$ -mercaptoethanol. Total protein content was determined by the Bradford technique (40). Creatine kinase isoenzymes were resolved on 1% agarose gels (Corning Medical, Medfield, MA) in Tris-barbital buffer. Electrophoresis was carried out at 4°C for 20 min at 170 V. The gels were overlaid with Rosalki agent (Corning Medical) with creatine phosphate (Boehringer Mannheim Biochemicals, Indianapolis, IN) as substrate, photographed under ultraviolet illumination, and analyzed by scanning densitometry (41). MM, MB, and BB denote the "muscle" homodimer, heterodimer, and "brain" homodimer, respectively.

**Cell number.** To determine cell number, myocyte cultures were incubated in 0.1% trypsin for 10 min (> 98% viable single cells). Mesenchymal cell cultures were incubated with trypsin for 12–15 min. Each plate was washed three times in phosphate-buffered saline to remove residual adherent cells, and complete removal of the cell populations was confirmed by phase-contrast microscopy. Cell counts then were obtained on the single cell suspensions using a hemocytometer, counting at least 200 cells for each of two replicate measurements of each independent sample.

**Statistical procedures.** Experimental results were compared by the unpaired two-tail *t* test and Scheffe's multiple comparison test for single factor analysis of variance, using a significance level of *P* < 0.05.

## Results

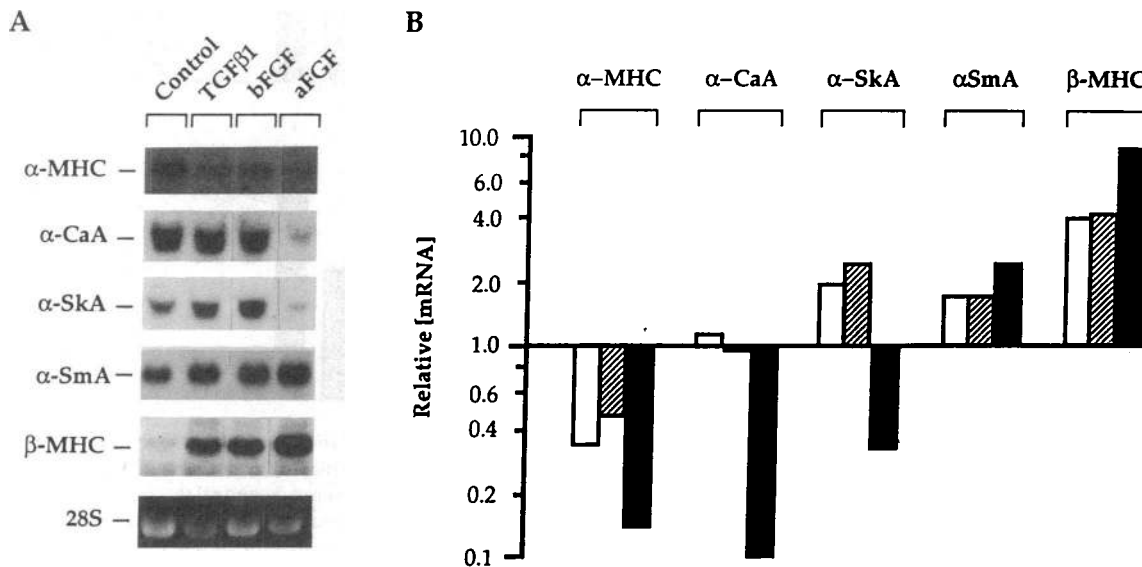
Cardiac myocytes plated at 10<sup>5</sup> cells/cm<sup>2</sup> formed a functional syncytium (100–140 contractions per min) within 24 h in serum-free medium. To characterize the extent of myocardial differentiation *in vitro*, actin and MHC gene expression were



**Figure 1.** Cultured cardiac myocytes possess differentiated phenotypic properties. (A) RNA blot hybridization was performed as described in Methods, and the relative hybridization signal of  $\alpha$ MHC and  $\alpha$ CaA to  $\beta$ MHC and  $\alpha$ SkA, respectively, is shown for cultured myocytes (solid bar) vs. the neonatal (2-d-old) rat heart in vivo (gray bar). Corresponding ratios for MHC and sarcomeric actins in the adult rat heart were at least 10 and 20, respectively. (B) The distribution of creatine kinase isoenzymes in cultured myocytes (solid bars) was comparable to that seen in the intact neonatal heart (gray bars). Corresponding values for the adult rat heart were: MM 60%, MB 27%, BB 13%.

analyzed by Northern hybridization after 48 h in serum-free medium and 24 h in serum-free medium supplemented with the diluent used for growth factor studies. As shown in Fig. 1 A, the relative hybridization signal for  $\alpha$ MHC vs.  $\beta$ MHC mRNA was about fivefold greater in cultured cardiac myocytes than in age-matched samples of neonatal rat heart in vivo (4.10:0.8). To exclude the possibility that the preferential expression of  $\alpha$ MHC was merely due to the concentration of thyroxine in the serum-free medium, steady-state levels of  $\alpha$ -actin mRNA also were analyzed. In overall agreement with the results for MHC gene expression, the relative intensity of  $\alpha$ CaA vs.  $\alpha$ SkA was about ninefold greater in the cultured cardiac myocytes than was found in the intact heart (6.47:0.670). Thus, the proportional expression of both  $\alpha$ MHC and  $\alpha$ CaA was augmented in the dissociated, cultured cells. As shown in Fig. 1 B, the distribution of creatine kinase isoenzymes expressed in culture was similar to that found in neonatal and adult ventricular muscle. Taken together, these data indicate that cultured cardiac myocytes possess differentiated phenotypic properties which are at least appropriate to the cells' developmental stage.

To investigate whether developmentally regulated gene expression in cardiac muscle cells might be susceptible to control by one or more specific growth factors, contractile protein gene expression was analyzed in ventricular myocytes cultured for 72 h as described above, after 24 h in the presence of TGF $\beta$ 1, bFGF, aFGF, or vehicle. Results in parentheses are shown relative to levels of expression in control cells. In partial agreement with their ability to suppress sarcomeric gene expression in skeletal muscle, TGF $\beta$ 1 and bFGF each inhibited expression of the "adult"  $\alpha$ MHC gene by > 50% (TGF $\beta$ 1, 0.34; bFGF, 0.47; Fig. 2). Conversely, each growth factor markedly stimulated the "fetal"  $\beta$ MHC gene approximately fourfold (TGF $\beta$ 1, 4.09; bFGF, 4.29). aFGF produced reciprocal changes in MHC expression qualitatively similar to, but greater than, those provoked by the other growth factors

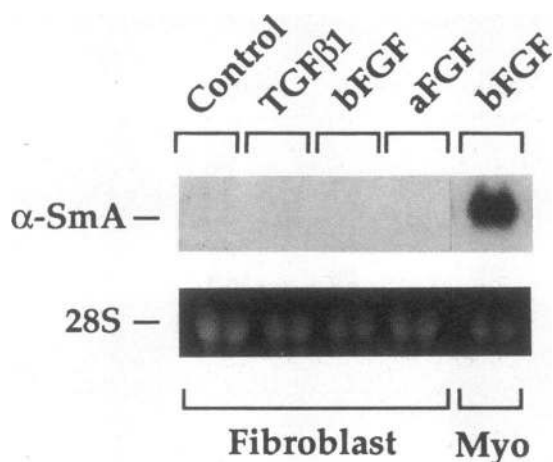


**Figure 2.** Peptide growth factors exert selective and differential effects on contractile protein gene expression in cardiac muscle. (A) Myocardial gene expression was analyzed by Northern blot hybridization in cardiac myocytes subjected to TGF $\beta$ 1, bFGF, or aFGF for 24 h. Ethidium-bromide stained 28S ribosomal RNA is shown for comparison. (B) Results for five contractile protein genes were quantitated by scanning densitometry and are shown relative to expression in control cells treated with serum-free medium and vehicle alone. (Open bar) TGF $\beta$ 1; (hatched bar) bFGF; (solid bar) aFGF.

( $\alpha$ MHC, 0.14;  $\beta$ MHC, 8.71). Whereas exogenous thyroid hormone prevents the transition between MHC isoforms after aortic constriction in vivo (3), all three peptide growth factors, at the concentration tested, produced down-regulation of  $\alpha$ MHC and up-regulation of  $\beta$ MHC despite the presence of 1 nM thyroxine in the medium.

All three peptide growth factors also provoked changes in  $\alpha$ -actin gene expression, which were distinct from the reciprocal regulation each produced in the MHC genes (Fig. 2). Whereas neither TGF $\beta$ 1 nor bFGF significantly altered  $\alpha$ CaA transcript availability (TGF $\beta$ 1, 1.17; bFGF, 0.97), both growth factors stimulated approximately twofold expression of the  $\alpha$ SkA gene, whose expression is associated with the embryonic or hypertrophic heart. Similar results were obtained in each of two independent experiments. Thus, in cardiac myocytes, four contractile protein genes exhibit a continuum of responses to TGF $\beta$ 1 and bFGF, and the effects evoked were highly concordant. In contrast, aFGF differed in its consequences for expression of these sarcomeric actin genes, and was a potent inhibitor of both  $\alpha$ CaA and  $\alpha$ SkA in cultured cardiac myocytes.

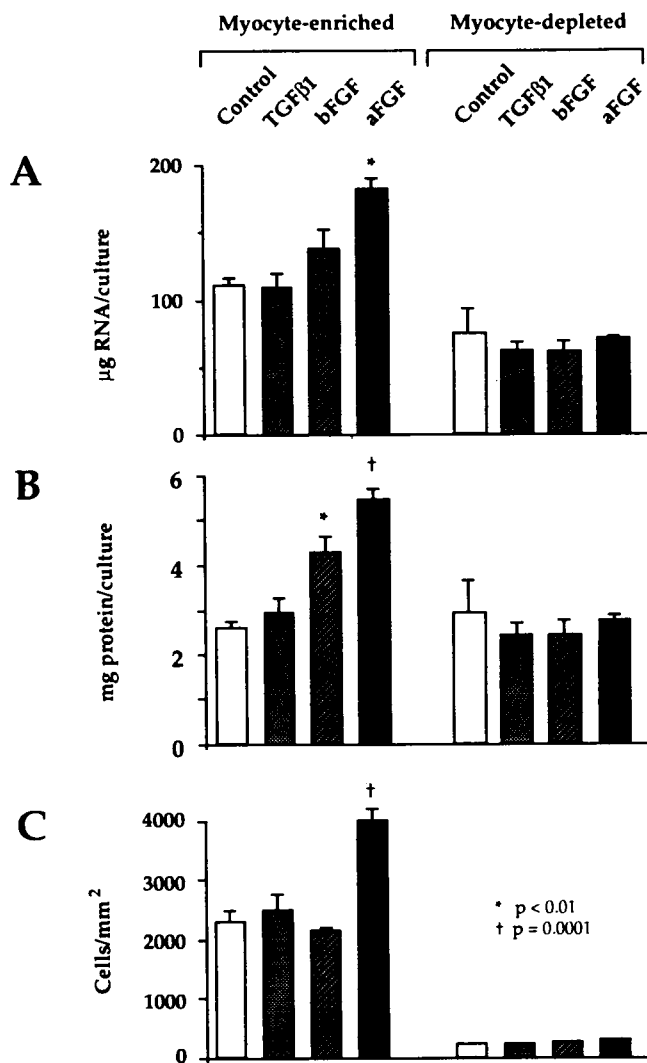
A third sarcomeric actin,  $\alpha$ SmA, is expressed in cardiac myocytes prior to the induction of either cardiac or skeletal actin (42). This ontogenic relationship among the  $\alpha$ -actin genes suggests two mutually exclusive hypotheses: that aFGF might suppress all three  $\alpha$ -actin genes or, alternatively, that it might selectively stimulate  $\alpha$ SmA, in the context of a phenotype even more primitive than that provoked by the other peptides. As shown in Fig. 2,  $\alpha$ SmA mRNA levels were increased by all three growth factors, and the greatest increase in  $\alpha$ SmA expression was induced by aFGF (TGF $\beta$ 1, 1.69; bFGF, 1.75; aFGF, 2.45). Neither basal expression of  $\alpha$ SmA nor its up-regulation by growth factors was detected in myocyte-depleted cultures enriched for cardiac mesenchymal cells (Fig. 3). Similarly, these growth factors did not induce  $\alpha$ SkA or  $\alpha$ CaA



**Figure 3.** Up-regulation of  $\alpha$ SmA gene expression in myocardial cell cultures does not entail its induction in fibroblastic cells. Myocyte-depleted (Fibroblast) cultures were incubated for 48 h in the serum-free medium, and treated for 24 h with diluent (control) or the peptide growth factors indicated.  $\alpha$ SmA gene expression was analyzed by Northern blot hybridization (cf. Fig. 2). For comparison, RNA isolated from cardiac myocytes treated with bFGF is shown at the right (Myo). Ethidium-bromide stained 28S ribosomal RNA is shown below.

in the fibroblastic cells (Parker, T. G., and M. D. Schneider, unpublished observations; cf. reference 26).

To examine the hypothesis that the differing effects of aFGF on contractile protein gene expression might be associated with distinct effects on myocardial growth in vitro, total RNA, protein content, and cell number were examined in myocardial cell cultures treated with each of the three peptides at the concentrations shown above (Fig. 4). RNA and protein content were analyzed in four and five independent experiments, respectively, and cell number determined in seven. The interval examined was limited, to avoid the potentially confounding effect of sustained fibroblast proliferation and the contingency that longer exposure to a given factor might alter



**Figure 4.** TGF $\beta$ 1, bFGF, and aFGF elicit dissimilar growth responses in myocardial cells. (A) Total cellular RNA, (B) protein, and (C) cell number. (Left) Myocyte-enriched and (right) myocyte-depleted cultures prepared from neonatal rat ventricles were cultured in serum-free medium for 48 h and treated for 24 h with vehicle or purified growth factors as described. The mean  $\pm$  SE is shown, except RNA and protein in myocyte-depleted cultures are mean  $\pm$  SD. *P* values are indicated for all comparisons with vehicle-treated control cultures where analysis of variance was significant by Scheffe's test (\**P* < 0.01; †*P* = 0.0001). (Open bar) control; (gray bar) TGF $\beta$ 1; (hatched bar) bFGF; (solid bar) aFGF.

the cells' growth responses as a consequence of dedifferentiation. In spite of its demonstrated effects on actin and MHC expression, TGF $\beta$ 1 did not increase RNA, protein, or cell number, in agreement with the fact that TGF $\beta$ 1 is a potent regulator of differentiated gene expression in skeletal muscle which exerts neither positive nor negative effects on myocyte growth (21, 22).

Although bFGF provoked changes in contractile protein gene expression similar both qualitatively and quantitatively to those produced by TGF $\beta$ 1, bFGF increased protein content at 24 h by 65% relative to control ( $4.27 \pm 0.36$  vs.  $2.59 \pm 0.16$   $\mu$ g per culture;  $P = 0.0029$ ). During the interval tested, no effect of bFGF on cell number was seen; differences in RNA content, similarly, were not statistically significant. These observations concur with evidence that bFGF increases cardiac myocyte number < 30% even after 7 d (43) and elicits mitotic growth in skeletal myocytes only in the presence of serum concentrations higher than those used here (18). In contrast, at 24 h, aFGF had stimulated protein content more than twofold ( $5.47 \pm 0.22$   $\mu$ g per culture;  $P = 0.0001$ ), increased total RNA by 46% ( $110 \pm 5.6$  vs.  $161 \pm 12.6$   $\mu$ g per culture;  $P = 0.0098$ ), and increased cell number  $\sim 75\%$  ( $2,290 \pm 180$  vs.  $3,980 \pm 318$  cells/mm $^2$ ;  $P = 0.0001$ ). To exclude a preponderant effect of aFGF and bFGF on the residual nonmuscle cells, confluent myocyte-depleted cultures were examined for comparison (cf. references 10, 27;  $n = 2$  for RNA and protein;  $n = 6$  for cell number). RNA and protein content in the myocyte-depleted "fibroblast" cultures were comparable to those in parallel myocyte-enriched cultures, despite differences in cell number per square millimeter. After 48 h in the same serum-free medium used for cardiac myocytes and 24 h of growth factor stimulation, neither RNA, protein, nor cell number in mesenchymal cultures differed from the control values (Fig. 4). Despite the potential for discordance between mesenchymal cells in the myocyte-depleted vs. myocyte-enriched cultures, these comparisons argue against the interpretation that the growth responses observed in cardiac "myocytes" occur chiefly or exclusively in the nonmuscle cells.

## Discussion

Successful application of in vitro methods to the molecular biology of cardiac growth has been impeded by the absence of permanent cell lines, by evidence that conventional cell culture methods fail to maintain a fully differentiated phenotype, and by the paucity of physiologically relevant agonists. The investigations reported here demonstrate that cardiac myocytes are, directly or indirectly, targets for the action of three peptide growth factors: TGF $\beta$ 1, bFGF, and aFGF. Differentiated ventricular muscle cells possessed complex, selective responses to both TGF $\beta$ 1 and bFGF: up-regulation of both "fetal" isoforms,  $\alpha$ SkA and  $\beta$ MHC; down-regulation of  $\alpha$ MHC expression; and little or no change in  $\alpha$ CaA. Thus, either peptide alone was sufficient to elicit a program of altered gene expression which diverges from the apparently uniform suppression found in skeletal muscle cells and strongly resembles events characteristic of pressure overload in vivo. Other ligands which alter contractile protein gene expression in cardiac myocytes fail to correspond so precisely to hypertrophy produced by load.

By contrast, aFGF inhibited the expression of the cardiac and skeletal actin genes in ventricular myocytes. Few, if any, qualitative differences have been reported previously in the

action of basic versus acidic FGF in a given cell lineage (44–46). Both aFGF and bFGF repress muscle creatine kinase in skeletal myoblasts (19) and, conversely, induce the conversion of ectoderm to mesodermal muscle progenitor cells in *Xenopus* embryos (47). Thus, the action of these growth factors is contingent on cell maturation, as well as lineage. Where differences in their potency have been examined, aFGF was 20- to 100-fold less active than bFGF (44, 46). Acidic FGF inhibits striated  $\alpha$ -actin gene expression even at 1.25 ng/ml, 20-fold lower than the concentration of bFGF tested here (Parker, T. G., and M. D. Schneider, unpublished results). In contrast,  $\alpha$ SmA mRNA levels were increased by all three growth factors. Studies utilizing the recombinant proteins would test the interpretation that the observed disparities between the action of acidic and basic FGF on cardiac myocytes are intrinsic to these peptides, and not the result of minor contaminants. The apparent association between proliferative growth and down-regulation of the striated  $\alpha$ -actin genes merits additional investigation. By comparison, aFGF failed to provoke growth in myocyte-depleted cultures. While anomalous responses are unlikely in fibroblasts which have been passaged only once, the possibility exists that growth properties differ subtly between cardiac fibroblasts in primary vs. secondary culture, and it therefore will be useful to examine growth regulation in low-density cardiac cultures, where cell identity can be more readily ascertained (10).

The present data are consistent with the previous observation that serum exerts multifunctional effects on cardiac growth and actin gene expression which vary with the myocytes' precise stage of differentiation (27), and suggest the testable hypothesis that the ability of ventricular muscle cells to respond to aFGF declines during the transition from hyperplastic to hypertrophic growth. The relative expression of acidic and basic FGF by cardiac myocytes may itself be developmentally regulated (15). As illustrated by disparities in the action of adrenergic agonists on ventricular muscle cells at different developmental stages (9, 48), the differentiated phenotype of neonatal cardiac myocytes after stringent mitogen withdrawal does not necessarily predict that adult cardiac muscle cells share all, if any, of the responses to growth factors exhibited by younger cells.

The precise role of peptide growth factors during hypertrophy in the intact animal or man also remains a matter of conjecture. For example, TGF $\beta$  suppresses the growth of endothelial cells in monolayer culture (49) but stimulates angiogenesis in vivo (50). Moreover, the possible complexities of autoregulation and cooperative interaction among these growth factors in the heart have not yet been explored. These unanswered questions may have particular interest in view of recent studies demonstrating both the existence of TGFs (14) and FGFs (15) in cardiac muscle cells during development, as well as the up-regulation of TGFs (16) and FGFs (51) after myocardial infarction or chronic ischemia. A role for cardiac growth factors during embryogenesis has been suggested in formation of the cardiac valves (52) and commitment of splanchnic mesoderm to the cardiac lineage (53), akin to the synergistic action of FGF and TGF $\beta$  analogues in skeletal muscle ontogeny (26, 47). TGF $\beta$ 1 (54) and the FGFs (44) are the prototypes for two multigene families of peptides with complex and diverse effects on cell growth, differentiation, and morphogenesis: homologues including TGF $\beta$ 3 and  $\beta$ 4 also may be expressed in cardiac muscle cells (55).

The findings reported here suggest the provisional hypothesis that peptide growth factors which exist in cardiac myocytes and their extracellular matrix may contribute to cardiac hypertrophy and the associated "fetal" phenotype, through autocrine or paracrine mechanisms. Peptide growth factors might also be expected to affect nonmuscle components of the myocardium, through fibroblast proliferation or accumulation of the extracellular matrix, and add to the interstitial fibrosis which is often a hallmark of pathologic hypertrophy. The clinical relevance of TGF $\beta$  for disease states and tissue repair has been recently reviewed (54). The selective and heterogeneous actions shown by these peptide growth factors also might contribute to topographic or temporal discrepancies in the appearance of fetal myosin and actin transcripts (56).

Finally, these results may provide evidence of previously unanticipated disparities between the program of differentiation in skeletal vs. cardiac muscle. In skeletal muscle, serum factors uniformly suppress all sarcomeric actin and MHC genes before irreversible differentiation (57, 58), and are not thought to modify muscle-specific gene expression once terminal differentiation has occurred (18, 20, 21, 57, 58). In cardiac myocytes, on the other hand, TGF $\beta$ 1 and bFGF selectively inhibited the "adult"  $\alpha$ MHC transcript, concurrent with up-regulation of all three fetal contractile protein genes examined thus far. This intricate set of events perhaps is most simply interpreted in the context of a lineage-specific action of TGF $\beta$ 1 and bFGF on ventricular myocytes. Alternatively, the use of coding-sequence probes (57, 58) may have obscured possible transitions among the highly conserved actin and MHC isoforms, in myotubes exposed to mitogenic medium. Despite the fact that muscle cell receptors for both TGF $\beta$ 1 (59) and FGFs (60) down-regulate dramatically upon terminal differentiation, L6E9 myotubes remain able to up-regulate the fibronectin and collagen genes after treatment with TGF $\beta$ 1 (20), as well as *c-myc* after exposure to serum (58). The myogenic determination gene MyoD1 is not constitutively expressed within the skeletal muscle lineage (cf. reference 34), but rather can be repressed by FGFs and TGF $\beta$ 1 (61) or other inhibitors of muscle differentiation (62, 63). Furthermore, the induction of myogenin itself accompanies growth factor withdrawal (36). Together, such observations are consistent with the inference that these nuclear proteins participate directly in the control of muscle-specific genes by growth factors. However, in cardiac muscle cells no protein with a corresponding structure and function has yet been identified. Cellular *ras* oncogenes produce a phenotype comparable to that provoked by TGF $\beta$ 1 (62–66) and suppress  $\alpha$ -actin gene expression in skeletal muscle cells (62, 64). It will be intriguing to determine whether activated *ras* alleles, by contrast, might selectively stimulate the transcription of  $\alpha$ SmA,  $\alpha$ SkA, or both when introduced into cardiac myocytes. Future attempts to interpret our results in the context of growth factor-inducible nuclear oncogenes and other ubiquitous transcription factors will need to account for the disparate effects of these peptide growth factors on myocardial gene expression.

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