Acidic and Basic Fibroblast Growth Factors in Adult Rat Heart Myocytes
Localization, Regulation in Culture, and Effects on DNA Synthesis

Edith Speir, Virginia Tanner, Ana Maria Gonzalez, James Farris, Andrew Baird, and Ward Casscells

Basic fibroblast growth factor (bFGF) and acidic fibroblast growth factor (aFGF) are involved in the induction of embryonic mesoderm, angiogenesis, neuronal differentiation, and proliferation and survival of many cell types. In cardiac myocytes their roles are not well understood. Effects of fibroblast growth factors on reexpression of fetal actin genes have been reported. In freshly isolated adult rat cardiac myocytes, bFGF mRNA was not detectable by in situ hybridization, although the cells contained significant amounts of bFGF and aFGF as quantified by radioimmunoassays, mitogen assays with immunoneutralization, and Western blotting. After culturing, bFGF mRNA was detected (aFGF mRNA was not studied), and the cells contained 2.5-fold more bFGF and 60% more aFGF than freshly isolated cells. The FGFs were not found in conditioned medium. They were localized, especially in cultured cells, to the nucleus. Cultured myocytes bound fourfold more 125I-FGF than freshly isolated cells and expressed the fibroblast growth factor R-1 (Rg) gene. The addition of bFGF or aFGF in serum-free medium to pure populations of myocytes (after 10 days in culture, at which time they are spread, beating, and multinucleated) led to increased thymidine incorporation. Expression of fibroblast growth factors and fibroblast growth factor receptors by adult cardiac myocytes that survive the shock and "dedifferentiation" of culturing may contribute to DNA synthesis and, by analogy, to other cell types, to regulation of ribosomal and actin genes, and to cell survival. These possibilities and their in vivo relevance will require further study. (Circulation Research 1992;71:251–259)

Key Words • heart-derived fibroblast growth factors • tissue • cardiocytes • nuclear fibroblast growth factors • postmitotic cells • fibroblast growth factor receptors

Acidic fibroblast growth factor (aFGF) and basic fibroblast growth factor (bFGF) have been identified in a variety of normal and malignant tissues and cells. Fibroblast growth factors (FGFs) are potent mitogens and survival factors for many cell types in vitro, and they stimulate the angiogenic response in vivo. bFGF can induce or delay differentiation and acts as a mesoderm-inducing morphogen.

Recently, we and others extracted aFGF and bFGF from normal human hearts, from bovine and canine hearts, from brain, and from freshly isolated adult rat cardiac myocytes. The roles of these factors in these nondividing cells are not known. It is known that proliferating skeletal myoblasts, when deprived of FGF in vitro, become irreversibly postmitotic, because of permanent loss of FGF receptors within hours after FGF withdrawal. In cultured neonatal cardiac myocytes, administration of bFGF and aFGF has different effects on DNA synthesis and expression of actin isoforms. When isolated rod-shaped adult cardiocytes are placed in culture, they spread and resume beating spontaneously after 3–5 days in culture, acquire multiple nuclei by DNA replication, but fail to complete mitosis. We hypothesized that new expression of FGFs in these myocytes might be one of the mechanisms of cell survival and cellular hypertrophy.

Materials and Methods
Myocyte Isolation and Culture

Calcium-tolerant myocytes were isolated using the perfusion technique of Claycomb and Palazzon and the attachment procedure of Lundgren et al. Isolated myocytes were counted with a hemocytometer, and 10,000–20,000 rod-shaped cells/cm² were plated in 35-mm dishes (Falcon) precoated with 20 µg/ml laminin (a gift from Dr. H. Kleinman, National Institute of Dental Research, NIH, Bethesda, Md.). One heart yielded 3–4 million rod-shaped cells; 5–10% of the plated rods typically attached within minutes, spread, and started beating spontaneously after 3–5 days in culture. At that time, more than 80% of the cells were binucleated, and some were multinucleated. Nonmuscle cells were eliminated from the cultures by adding 2.25 µg/ml cytosine-1-β-d-arabinofuranoside hydrochloride (ara-C) (United States Biochemical Corp., Cleveland, OH) to the cultures.

From the Cardiology Branch (E.S., V.T., W.C.), National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Md., and the Whittier Institute (A.M.G., J.F., A.B.), La Jolla, Calif.
Address for correspondence: Edith Speir, BS, National Institutes of Health, Building 10, Room 7B-15, Bethesda, MD 20892.
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Ohio) for the first 4 days of culture. Cardiocytes were harvested by washing twice with cold Puck’s saline, adding 1 ml of 1 M NaCl, 0.01 M Tris (pH 7.4), and proteinase inhibitors to each dish, and using a cell scraper to obtain 0.5–1×10⁶ million cells in 10 ml of this buffer. The cells were then dispersed by a freeze–thaw cycles, homogenized, and centrifuged at 48,000g for 30 minutes, and the clear supernatant was adjusted to 0.5 M NaCl (cell lysate). Freshly isolated cardiocytes were washed in Joklik’s medium (five changes of medium) and counted by hemocytometer; 0.5–1×10⁶ cells were resuspended in 10 ml of 1 M NaCl extraction buffer and processed as described for the cultured cells. Results were normalized for cell number.

Quantitation of aFGF and bFGF

The contents of aFGF and bFGF in freshly isolated versus cultured cardiocytes were compared in 1 M (aFGF) and 1.5 M (bFGF) fractions from heparin-Sepharose chromatography using the 3T3 cell assay as described with confirmation by radioimmunoassay (RIA), immunoblotting, and neutralization studies (see below).

Competitive RIA

For quantification of bFGF we used human recombinant ¹²⁵I-bFGF as a ligand and anti-bFGF immunoglobulin (Ig) G “cat” as the binder. The RIA for aFGF was developed in our laboratory (S. Shrivastav, manuscript in preparation) with antisera raised against a synthetic peptide corresponding to aFGF residues 50-82. Human recombinant aFGF was radiiodinated by iodogen (Pierce Chemical Co., Rockford, Ill.) with the modification of adding 0.5 µg/µl sodium heparin (The Upjohn Co., Kalamazoo, Mich.) to the reaction mixture. After that, the procedure follows the one published for the bFGF RIA.

Western Blot Analysis

Heparin-Sepharose column fractions (1.1 M or 1.5 M NaCl) with peak mitogenicity were concentrated and desalted by centrifugation in Centricon-10 concentrators (Amicon, Beverly, Mass.). Aliquots were subjected to sodium dodecyl sulfate–10–20% polyacrylamide gel electrophoresis, and the proteins were transferred to nitrocellulose with the semidy Polyblot system. We used the following seven polyclonal and two monoclonal antisera: anti-aFGF residues 7-16 or 50-82 and anti-bFGF residues 33-43 or 135-145 (gifts from Dr. J. Sasse, Shriners Hospital for Crippled Children, Tampa, Fla.). Monoclonal anti-aFGF was purchased from Upstate Biotechnology Inc., Lake Placid, N.Y.; anti-bFGF “dog” (a gift from Dr. D. Gospodarowicz, University of California, San Francisco), anti-bFGF 1-24 and 967, and monoclonal anti-bFGF antibody 78, directed to the first nine residues of human recombinant bFGF (a gift from Takeda Ltd., Japan) were all at dilutions of 1/1,000 except anti-bFGF 1-24, which was diluted 1/10,000.

Immunohistochemistry

Freshly isolated adult rat cardiac myocytes were attached to two-chamber culture dishes (LabTek) coated with 20 µg/ml laminin. After incubation at 37°C with 5% CO₂ for 4 hours (20,000 cells/cm² in 1 ml Joklik’s medium), cells were fixed and permeabilized with 0.2% saponin. The cells were then incubated overnight with primary antibody in phosphate-buffered saline (PBS) with 2% goat and 1% rat serum at 4°C with gentle rocking. Cultured myocytes were fixed while beating (10–18 days after plating) and processed as described for freshly isolated cells. We used the anti-aFGF or anti-bFGF antisera described under “Western Blot Analysis” and several controls described in detail elsewhere.

Salt hearts were fixed by perfusion with 10% formalin. Paraffin sections (6 µm) were prepared for immunostaining as described. Serial sections were incubated with either anti-bFGF 1-24, anti-bFGF “dog,” or anti-aFGF 50-82 or 7-16 at 4°C overnight, all at dilutions of 1/1,000. Controls were incubated with nonimmune serum or IgGs or with immune sera after the tissue sections were washed with 2 M NaCl to remove bFGF associated with heparin sulfate.

In Situ Hybridization

Freshly isolated rod-shaped myocytes attached to laminin for 4 hours at 37°C or spreading myocytes maintained in two-chamber glass slides (Thomas Scientific) for 5 or 14 days were washed three times with PBS and fixed with 2% paraformaldehyde in PBS for 15 minutes. The in situ hybridization was performed as described before. Cells were incubated with labeled sense or antisense RNA, washed, coated with photographic emulsion, exposed for 3 weeks at 4°C, and developed. The slides were then examined by fluorescence and dark field microscopy. Hybridization was evaluated arbitrarily by counting grains (signal) localized over the cell body. Forty slides were evaluated. The experiment was repeated once.

Effect of aFGF and bFGF on DNA Synthesis

Rod-shaped myocytes (5×10⁵) were plated into 6-cm Falcon dishes. After 10–14 days in culture, cells were washed and incubated with serum-free Eagle’s essential medium in Earl’s balanced salt solution (EMEM) for 24 hours. aFGF or bFGF (1 or 10 ng/ml [³H]thymidine (20 Ci/mmol) were added to the cells. One set of dishes received this treatment once; 24 hours later, cells were washed with cold Puck’s saline, gently scraped, and briefly centrifuged, and the resultant pellet was sonicated in 100 µl PBS. Aliquots were used for protein estimation by Quantigold (Diversified Biotech, Newton Center, Mass.) and for the determination of [³H]thymidine incorporation. Another set of dishes received three doses of FGF and [³H]thymidine. Human recombinant aFGF and bFGF were generous gifts from Drs. L. Coussens and P. Barr, Chiron Corp.

Single-Cell Autoradiography

Myocytes cultured in chamber slides (glass) for 14 days were rinsed twice with PBS and once with EMEM and 25 M EMES (pH 7.4) and incubated for 1 hour at 22°C with 100 pM ¹²⁵I-aFGF in EMEM, 25 M EMES, and 0.15% gelatin (binding buffer) in the presence of absence of 1 µg/ml unlabeled bFGF. The cells were then fixed with 2% glutaraldehyde, rinsed with H₂O₂, and coated with Kodak NTB2 emulsion for 10 days at 4°C.
Binding of 125I-aFGF to Myocytes

Cells in 3 ml EMEM, 25 mM HEPES, and 12% fetal bovine serum were plated at 10^5 per 6-cm dish. Under these conditions, myocytes attach within 1 hour after plating and maintain their original rod-shaped morphology for 24 hours in culture. Binding studies were done 12 hours or 14 days after plating the cells. After rinsing the dishes with PBS and then with binding buffer, 200 pM 125I-aFGF (in the presence or absence of 1 μg/ml unlabeled bFGF) in 2 ml binding buffer was added for 1 hour. The medium and the following buffers were removed to counting vials: two washes with PBS, two washes with 1 M NaCl and 20 mM HEPES (pH 7.4), and finally two washes with 1 M NaCl and 20 mM sodium acetate (pH 4.0).20

Results

Mitogenicity of Cardiac Tissue or Myocyte Extracts: Cultured Myocytes Contain More FGF-like Mitogenic Activity Than Freshly Isolated Myocytes

The crude myocyte extracts caused a twofold increase in [3H]thymidine incorporation by 3T3 cells. Heparin-bound 1.1- or 1.5-M fractions also had mitogenic activity (Figure 1). Biological activity eluted from the columns as two peaks at 1.1 and 1.5 M, characteristic of aFGF and bFGF, respectively. Figure 1A illustrates a twofold increase of activity in 10-day cultured myocytes when compared with freshly isolated cells (Figure 1B).

RIAs

Cultured myocytes yielded twofold to threefold more bFGF and 60% more aFGF than freshly isolated cells (Figures 1A–1C). 3T3 growth factor units calculated from serum and FGF standard curves and the corresponding RIA results are summarized in Table 1. Intra-assay and interassay variability was ±10% and ±15%, respectively. To address the possibility that the column fractions contained other heparin-binding growth factors besides bFGF and aFGF, we performed neutralizing studies4 and found that anti-bFGF antibodies neutralized 90% of the mitogenic activity of the 1.5-M fraction and anti-aFGF antibodies neutralized 90% of the activity in the 1.1-M fractions (not shown). Thus, aFGF and bFGF account for almost all the heparin-binding mitogenicity of cardiac myocytes.

Western Blotting

We used an extraction buffer containing 1 M NaCl (pH 4.5) and proteinase inhibitors, which consistently gave good yields of 17-kd bFGFs and, less consistently, 17-kd aFGFs. aFGF often migrated as a 15-kd band (not shown) or as a 15–16-kd doublet in the sodium dodecyl sulfate gel. Both aFGF and bFGF were immunoblotted from the lysates of freshly isolated or cultured myocytes, using two or three different antibodies, with similar results (not shown).

Immunolocalization of aFGF and bFGF in Freshly Isolated and Cultured Myocytes

Immunoperoxidase reaction product to aFGF was seen in freshly isolated rod-shaped myocytes with three different antibodies: one directed against the amino terminus (region 7–16, not shown), one to the midsection (region 50–82, Figures 2B and 2C), and one monoclonal antibody raised to the whole molecule (bovine aFGF, not shown). In Western blots, the antibodies were monospecific in recognizing aFGF in fresh and cultured myocytes (not shown). There appeared to be more intense reactivity in the plasma membrane, in the nuclei, and in the cross-striations of the sarcomeres. When cells were exposed to immune reagents without being permeabilized first, there were a few spotty stains or no staining; however, extracellular FGFs could have been removed during enzyme digestion.

A very similar pattern was seen for bFGF (Figures 2A and 2D), using anti-bFGF 1–24 and anti-bFGF monoclonal antibody 78 (not shown). Myocytes showed lack of reactivity when treated with the following controls:

![Graph](https://example.com/graph.png)

**Figure 1.** Graphs showing results of heparin-Sepharose affinity chromatography of freshly isolated and cultured adult myocyte lysates and whole rat hearts analyzed for content of acidic fibroblast growth factor (aFGF) and basic fibroblast growth factor (bFGF) by radioimmunoassay and mitogen assays. Panel A: Extracts of 10^6 cultured cells were batch-adsorbed overnight on heparin-Sepharose, transferred to a 10-ml column, and eluted with a step gradient of NaCl as shown by the arrows. Aliquots of column fractions (1–5 μl) were added in triplicate to quiescent Balb/c 3T3 cells in 96-well plates as described. Open circles denote thymidine incorporation by 3T3 cells. Triangles denote aFGF radioimmunoassay values; solid circles denote bFGF radioimmunoassay values. Values represent the means of triplicate determinations (SD±10%) with background (5,000–7,000) or nonspecific (∼5%) counts subtracted. Construction of standard curves (not shown) using human recombinant bFGF and human recombinant aFGF in the 3T3 assay allowed for conversion of counts per minute into bFGF and aFGF values nearly identical to those generated by radioimmunoassay. Panel B: Lysates of 10^6 freshly isolated cells were processed as described in panel A. Aliquots (4 and 10 μl) were examined by aFGF or bFGF RIA. Radioimmunoassays and mitogen assays each demonstrate that freshly isolated cells have less aFGF and less bFGF than cells (of equal size and protein content) cultured for 10 days. Panel C: Rat heart tissue (10 g) frozen and pulverized in liquid N_2 was extracted with 1 M NaCl and proteinase inhibitors and then processed as described for the cells. Peak radioimmunoassay values coincide with peak mitogenicity.
immune IgG preadsorbed with the peptide used to raise it (Figures 2E and 2F), FGF-depleted IgGs, and non-immune rabbit sera (not shown), all at the same concentrations as the primary antibody.

When stained with anti-aFGF 50-82 or monoclonal anti-aFGF antibody 2G or I after 12–15 days in culture, myocytes again showed intense reaction product in the nuclei and the perinuclear zone. Similarly, examples of cultured myocytes (12–15 days) immunostained for bFGF with anti-bFGF 1-24 and 967 are shown in Figures 2J and 2L, respectively. Comparable results were obtained with the anti-bFGF monoclonal antibody. Use of FGF-depleted IgG (Figure 2K), nonimmune rabbit IgG, or omission of primary antibody resulted in no immunoreactivity (not shown).

**Immunolocalization of aFGF and bFGF in Rat Heart Tissue**

In 6-μm sections of formalin-fixed hearts of normal 7-week-old male rats, we found bFGF in myocytes, endothelial cells, and fibroblasts but little aFGF or bFGF in smooth muscle cells. The nuclei of a few cardiac myocytes were stained. It was not possible to determine by light microscopy whether the basement membrane of myocytes was immunoreactive for aFGF or bFGF (Figures 2M and 2O). One of the control myocytes is shown (Figure 2N): anti-aFGF 50-80 depleted by aFGF-Sepharose. In cryostat sections that were fixed in 1% formalin for 10 minutes, bFGF immunoreactivity was apparent in most cardiac myocyte nuclei and intercalated discs (not shown). The pattern of aFGF immunoreactivity was similar to that for bFGF.

**In Situ Hybridization**

Figure 3 shows examples of the results of in situ hybridization for bFGF mRNA. When examined by fluorescence microscopy, myocytes are autofluorescent (Figure 3, left panels). Freshly isolated myocytes have only background grains for both sense and antisense exposure (panels B and D). Cells cultured for 5 days have a strong signal (panel H) compared with background (panel F), and cells cultured for 10 days also have increased labeling when treated with antisense versus sense probe (panels L versus J). These results support our finding of increased levels of bFGF-like activities in cultured versus freshly isolated cells. Fifty to one hundred cells per 4-cm² chamber slide were hybridized. The background signal was not higher than 10 grains per square centimeter on negative control profiles, and hybridization was considered positive with 20 or more grains per square centimeter on the cell body. More than 70% of the rods were negative, and more than 80% of the cultured cells were positive. Figure 4 illustrates in situ hybridization of an flg (FGF receptor 1) probe to freshly isolated and spreading myocytes maintained in culture for 5 or 10 days. Freshly isolated cardiomyocytes express little flg mRNA, and although receptor expression is still low in the 5-day cells (Figure 4h), it is clearly higher in the cells cultured for 10 days (Figure 4I). More than 70% of the 10-day cells treated with antisense probes were positive (20 or more grains per square centimeter on the cell body).

**Effect of FGFs on DNA Synthesis**

When adult myocytes were fed with 1 or 10 ng/ml aFGF and 2 μCi/ml [3H]thymidine for 24 hours, DNA synthesis significantly increased by 75–85% over control as assessed by liquid scintillation counting in counts per minute per microgram protein. The effect was maximal with 1-ng doses of aFGF (Figure 5A). The response to identical doses of bFGF was virtually the same (Figure 5A, bars 3 and 4). When cells were fed with three doses of FGFs at 24, 72, and 120 hours in culture (panel 5B), there was a trend of a greater effect (when compared with one dose, Figure 5A). The control values are approximately 300 cpn after one dose of [3H]thymidine (Figures 5A and 5C) and approximately 1,000 cpn after three doses (Figures 5B and 5C).

**Single-Cell Autoradiography**

When myocytes maintained in chamber slides (glass) for 48 hours (Figure 6A), 5 days (Figure 6B), or 14 days (Figure 6D) were kept in serum-free medium for 24 hours, then incubated with freshly iodinated 125I-aFGF, and processed as described in “Materials and Methods,” cells showed significant label outlining the various shapes of the myocytes. The rod-shaped cell (panel A) that spreads in typical fashion from the short ends of the cylinder shows labeling at the spreading “ruffles” and perhaps at the cell membrane. The label was competed with 500-fold (5 μg/ml) excess unlabeled bFGF (Figure 6C). This indicates that the binding is specific and adds support to the indirect evidence of receptors in the binding study (see below).

**125I-aFGF Binding to Intact Myocytes**

We modified the method of 125I-aFGF binding to the FGF receptor in sparse cells in the monolayer. In the present study, the 2 M NaCl wash dislodged the freshly isolated myocytes from the dish and also removed a significant number of the cells that had spread for 10 days. We successfully used 1 M NaCl (pH 4.0) and immediately removed it, processing only one dish at a time for a total of nine dishes in one experiment. Results show a fivefold increase in bound 125I-aFGF (removed from the cells at pH 4.0) for myocytes maintained in culture for 10 days (24±6 fmol/10⁶ cells) versus freshly isolated cells in culture for 12 hours (6±1.7 fmol/10⁶ cells, p<0.01), at which time they have attached but have not spread. Nonspecific binding (20%) was assessed in parallel cultures in the presence...
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Figure 2. Photomicrographs showing immunoperoxidase identification of acidic fibroblast growth factor (aFGF) and basic fibroblast growth factor (bFGF) in freshly isolated (panels A–F) and cultured (panels G–L) myocytes from adult rat heart and normal rat heart (panels M–O). Cells were fixed with paraformaldehyde and permeabilized with 0.2% saponin–phosphate-buffered saline. Shown are the results obtained with anti-bFGF 1-24 (panels A, D, J, and O) and anti-bFGF 967 (panel L); lack of immunoreactivity is apparent when cells or tissue were incubated with anti-bFGF 1-24–depleted antibodies (panels E and F). All other controls were similar. Examples of staining with anti-aFGF monoclonal antibody (raised against bovine aFGF) (panel I) and with anti-aFGF 50-82 (panels B, C, G, and M) are shown. Three more controls are shown: incubation with anti-aFGF 50-82–depleted antibodies (panels H and N) and anti-bFGF 967 (panel K) preadsorbed human recombinant bFGF for 24 hours at 4°C in the presence of 1% crystalline bovine serum albumin in phosphate-buffered saline and passed through a small heparin column. The column flowthrough was used in place of primary antibody (panel K). Mouse myeloma immunoglobulin G (Cappel Research Reagents, Durham, N.C.) was used as a control for monoclonal antibodies. Magnifications, ×237.5 for panels A, C, and E; ×475 for panels B, D, and F; ×95 for panels G, H, J, and K; ×90.25 for panel I; ×118.75 for panel L; and ×209 for panels M, N, and O.
FIGURE 3. In situ hybridization for basic fibroblast growth factor mRNA. In the left panels, freshly isolated cells (panels A and C) and cells cultured for 5 days (panels E and G) or 10 days (panels I and K) were visualized with fluorescence microscopy (autofluorescence, no staining). Two cells have four nuclei (panels A and G), one cell has 3 nuclei (panel E), and the others are binucleated. The corresponding right panels show the identical cell in dark field photography. Four consecutive photomicrographs represent one set: freshly isolated cells (panels A–D), incubation with the sense strand (panels A and B), and incubation with the antisense strand (panels C and D). The 5-day culture is shown in panels E–H. No signal is seen in the control incubated with the “sense” strand (panels E and F). Hybridization is apparent in the high density of grains in three of the four nuclei (panel H). The 10-day-old myocytes had no label when treated with the sense strand (panel I), but dense grains are seen over both nuclei with the antisense probe (panel L).

FIGURE 4. In situ hybridization for flg (fibroblast growth factor R-1) mRNA. The left panels are photomicrographs of autofluorescent myocytes. The right panels are corresponding dark field views. Panels b, f, and i are labeled with the sense probe; panels d, h, and l are labeled with the antisense strand. When treated with antisense probe the freshly isolated cell (panel D) and the cell cultured for 5 days (panel H) show a weak signal, whereas the 10-day cell has a higher expression of flg mRNA. Magnification, ×165.

of 1 μg/ml unlabeled bFGF. Companion dishes were harvested and counted at the time of the binding study (not shown).

Discussion

FGFs have been isolated from cardiac tissue by several groups.4–7,21–23 In addition, FGFs were identified in adult myocytes in vivo8 and in neonatal myocytes in
and label change. Again, aFGF (bars replaced was not incorporated ['Hlthymidine acid after 48 hours in culture, cardiac myocytes express mRNA for bFGF, synthesize FGFs, contain more FGFs than freshly isolated cells, and express FGF receptors. The proteins are cell associated and are not detectable in myocyte-conditioned medium.

The increased synthesis of bFGF, aFGF, and their receptors could have several functions. The possibility of a mitogenic function is supported by the increase in thymidine incorporation that we and others observed in response to adding bFGF to the medium and by the fact that the activation of FGF synthesis just precedes the appearance of multinucleation. However, no increase in cell density was observed, in agreement with all prior studies.

23,24 Some groups detected aFGF only23,24; others showed the presence of bFGF only21,22. We have consistently found both aFGF and bFGF in cardiac tissue and cells. The failure to detect aFGF in several studies may be due to the fact that aFGF is more susceptible to degradation25 than bFGF and, most important, that sensitive anti-aFGF antibodies have only recently been available.

This is the first report, however, on the subcellular localization of FGFs in the adult cardiocyte and on the increased production and intracellular content of FGFs and FGF receptors when myocytes are placed in culture. In contrast, adult skeletal myocytes are incapable of binding FGFs even after culturing.10

Adult cardiocytes, though irreversibly postmitotic, are capable of further hypertrophy in response to increased pressure or work. The degree to which hypertrophy involves not only increased RNA and protein synthesis but also DNA synthesis and polyploidy is not well established, but it is known that cultured adult cardiocytes synthesize DNA and reexpress fetal contractile proteins.

The results of the present study indicate that, after being placed in culture, cardiac myocytes express mRNA for bFGF, synthesize FGFs, contain more FGFs than freshly isolated cells, and express FGF receptors. The proteins are cell associated and are not detectable in myocyte-conditioned medium.

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21,22,24,10

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FIGURE 5. Bar graphs showing that ['H]thymidine incorporation into DNA was increased when myocytes cultured for 10 days were fed with basic fibroblast growth factor (bFGF) or acidic fibroblast growth factor (aFGF). Panel A: Increased ['H]thymidine incorporation into cells that received 2 μCi/ml label and one dose of either aFGF (bars 1 and 2) or bFGF (bars 3 and 4). The higher (10 ng/ml) doses (bars 2 and 4) were not significantly different from the lower (1 ng/ml) doses (bars 1 and 3). Panel B: The effect of three equal doses of aFGF (bars 1 and 2) or bFGF (bars 3 and 4). The medium was replaced at 24, 72, and 120 hours; fresh fibroblast growth factor and label (2 μCi/ml) were added after each medium change. Again, maximal effects were reached with 1 ng/ml of aFGF or bFGF.

FIGURE 6. Single-cell 125I–acidic fibroblast growth factor autoradiography. Myocytes after 48 hours (panel A), 5 days (panel B), and 10 days (panel D) in culture show increasing intensity of radioligand binding. After 48 hours in culture, the cell spreads in typical fashion from the small end of the rod; the edges of the “ruffle” bind fibroblast growth factor. The membranes of the long axes of the rod are labeled; this is more clearly seen in a dark field exposure (not shown). The 5-day cell is clearly labeled, and the 10-day cell is intensely labeled. The binding of 125I–acidic fibroblast growth factor was competed with 500-fold excess unlabeled basic fibroblast growth factor (panel C).
bFGF also has been shown to be a survival agent, and the FGFs in myocytes may also function in some way to promote survival, delay senescence, regulate migration, and influence the production and degradation of the extracellular matrix. The induction of bFGF synthesis by cell culture may thus be similar to the wound response. Finally, bFGF, which delays the development of differentiated contractile proteins in skeletal myocytes, has recently been reported to differ from aFGF in its effects on α-actin expression, at least in neonatal cardiac myocytes: whether either FGF regulates actin or myosin isoforms in adult cells is not known.

The possible stimuli for increased production of aFGF, bFGF, and their receptors are not known. It is interesting to speculate, however, that the effects are mediated by the enzymatic digestion used in preparation of the cells, which leads to loss of matrix and cell–cell contacts. Another difference is that cells in vivo are exposed to plasma and those in vitro are exposed to serum. We cannot exclude roles for laminin (required for attachment) or even for ara-C (required to prevent fibroblast overgrowth), an important antimetabolite that is most cytotoxic to cells in the S phase. However, the increase in bFGF mRNA expression persisted until at least day 10, 5 days after the ara-C was washed out. Moreover, we have found a similar culture-induced increase in FGF expression in vascular endothelial and smooth muscle cells that were not exposed to ara-C or laminin.

Nuclear Immunoreactivity for bFGF and aFGF

The possibility of an intracellular role for bFGF and aFGF is suggested by our finding of specific immunoreactivity for these peptides in myocyte nuclei. In other cell types, evidence of nuclear FGF has been obtained recently by immunocytochemistry and by Western blotting of heparin-bound fractions of isolated nuclei. Although the difficulty of obtaining large numbers of cultured adult cardiocytes precludes analysis of nuclear extractions, specific immunostaining suggests the presence of both aFGF and bFGF in myocyte nuclei. These FGFs are presumably of myocyte origin, since these cells were never exposed to exogenous FGF. Whether these FGFs were first secreted and then taken up and transported to the nucleus is not known.

Bouche et al. showed by immunofluorescence that exogenous bFGF can be detected in both the nucleus and in the nucleolus of cultured endothelial cells and that it stimulates RNA polymerase I activity. The nuclear forms of bFGF are high molecular weight (21–24-kd) products of alternative translation start sites and are methylated. Their overexpression immortalizes endothelial cells.

References

20. Moscatelli D, Quarto N: Transformation of NIH 3T3 cells with basic fibroblast growth factor or the hst/k-fgf oncogene causes down-regulation of the fibroblast growth factor receptor: Reversal of morphological transformation and restoration of receptor numbers by Suramin. J Cell Biol 1989;109:2519–2527
24. Weiner HL, Swain JL: Acidic fibroblast growth factor is expressed by cardiac myocytes in culture and the protein is localized to the extracellular matrix. Proc Natl Acad Sci U S A 1989;86:2683–2687


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