Basic and Acidic Fibroblast Growth Factors Interact with the Same Cell Surface Receptors*

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Despite quantitative differences, the activity of basic and acidic fibroblast growth factors (FGF) on a wide variety of normal diploid cells derived from neuroectoderm and mesoderm is intrinsically similar. This suggests that they bind to the same cell surface receptors. This was investigated using a baby hamster kidney cell line (BHK-21) as a model. BHK-21 cell membrane components that interact with basic and acidic FGF have been identified by covalent cross-linking to their respective 125I-labeled ligands. Under appropriate conditions, basic and acidic 125I-FGF were cross-linked, using disuccinimidyl suberate, to two receptor species with apparent molecular masses of 145,000 and 125,000 daltons, respectively. The labeling of those receptors is inhibited when either native basic or acidic FGF are present in excess during incubation of cells with either basic or acidic 125I-FGF. Competition of basic 125I-FGF with increasing concentrations of native acidic FGF results in a preferential decrease in the labeling of the 125,000-dalton species, whereas competition of acidic 125I-FGF with increasing concentrations of native basic FGF leads to a preferential decrease in the labeling of the 145,000-dalton species. The data suggest that qualitatively both mitogens interact with the same 145,000- and 125,000-dalton receptor species. The different affinities displayed by acidic and basic FGF toward their common receptor molecules could explain why acidic FGF, depending on the cell type considered, is 20-100-fold less potent than basic FGF.

Basic fibroblast growth factor is a mitogen originally isolated from bovine brain and pituitary (1). It is also present in kidney, placenta, corpus luteum, adrenal gland, retina, macrophages, and chondrosarcoma (reviewed in Refs. 2 and 3). It has been shown to be mitogenic and to support the differentiation of a wide variety of neuroectoderm- and mesoderm-derived cells (4), in particular that of vascular endothelial cells (5). Related to basic FGF is acidic FGF, which has only been reported to be present in neural tissue (6). It is mitogenic and supports the differentiation in vitro of the same cell types as basic FGF (3, 7).

The available data have suggested until now that basic and acidic FGF are two different molecular entities. Acidic FGF has a pI ranging from 5.0 to 5.9 (3, 6) and a M₄ of 14,000-15,000 (3, 6, 7). Basic FGF has a pI of 9.6 (8) and a M₄ of 16,000 for the native molecule (8) and 14,500 for des-15-FGF (2, 3). The amino acid composition of basic and acidic FGF also apparently differs (6, 7, 8). Those differences, however, may not be as significant as one would have initially believed. Although acidic FGF is 30-100-fold less potent than basic FGF, both mitogens display a remarkable similarity in their biological activity toward endothelial and other mesoderm-derived cells (3, 7). Both mitogens also bind strongly to heparin, suggesting that their heparin binding domains could have a close structural homology (3, 7, 9). Comparison of the amino-terminal amino acid sequence of bovine brain basic and acidic FGF has revealed a striking homology between residues 14-38 of basic FGF and residues 4-29 of acidic FGF (3). The fact that antisera raised against a synthetic peptide from basic FGF, Tyr⁷⁸-FGF 69-87-NH₂, cross-react (2) with acidic FGF strongly suggests that additional sequence homologies exist between the mid portions of these two proteins (2).

Since both basic and acidic FGF are mitogenic for the same cell types, it raises the possibility that both molecules could interact with the same cell surface receptors. In the present study, we have addressed the problem of identifying specific receptor structures for basic and acidic FGF in the baby hamster kidney (BHK-21) cell line and have looked at the specificity of interaction of either basic or acidic FGF with those receptors. The data obtained indicate that both basic and acidic FGF do interact with the same receptor species.

EXPERIMENTAL PROCEDURES

Materials—JOIO-GEN and disuccinimidyl suberate were from Pierce Chemical Co. Heparin-Sepharose was from Pharmacia. Na125I was obtained from Amer sham Corp. Cellulose-acetate filters (GA-8) were from Gelman. Reagents used for NaDodSO₄-polyacrylamide gel electrophoresis and high and low molecular weight protein markers were from Bio-Rad.

Cells—BHK-21 cells were routinely maintained in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium (DF medium) supplemented with 5% calf serum, 2 mM glutamine, 0.25 μg/ml Fungizone, and 50 μg/ml gentamicin. For the comparison of the biological activity of basic and acidic FGF, BHK-21 cells were seeded at low density (4 × 10⁴ cells/35-mm dish) on gelatinized dishes and exposed to 2 ml of DF medium supplemented with 2 mM glutamine, 0.25 μg/ml Fungizone, 50 μg/ml gentamicin, 1 μg/ml transferrin, and 250 μg of protein/ml of high density lipoproteins (HDL). Increasing concentrations of either basic or acidic FGF were added every other day. On Day 4, triplicate dishes were trypsinized and cell number was determined using a Coulter counter (10).

Growth Factors—Basic and acidic FGF were purified from bovine pituitary and bovine brain as previously described (7, 9). EGF was purified from mouse submaxillary glands as described by Savage and Cohen (11).

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The abbreviations used are: FGF, fibroblast growth factor; HDL, high density lipoproteins; HEPES, N,N'-bis(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid; NaDodSO₄, sodium dodecyl sulfate; PBS, 0.01 M sodium phosphate, pH 7.4, 0.9% NaCl.

5631
Iodination of Basic and Acidic FGF—Basic FGF was iodinated as previously described (12). Acidic FGF (4 μg in 20 μl of 10 mM Tris-HCl, pH 7.0, 1 mM NaCl, 30 μl of water, and 60 μl of 0.2 M sodium phosphate, pH 7.2, were added to an IODO-GEN-coated glass tube (12). Na[125]I at a 2 M excess over acidic FGF was then added, and the mixture was incubated for 15 min at room temperature. At the end of the incubation, 100 μl of water, 60 μl of 0.1% sodium metabisulfite, and 30 μl of 0.1 mM KI were added to the tube. After 1-min, the contents of the tube were loaded on a heparin-Sepharose column (0.1 ml) that was pre-equilibrated with a buffer containing 20 mM sodium phosphate, pH 7.2, and 1.5 M NaCl. The iodinated preparations were routinely analyzed for structural homogeneity by NaDodSO4-polyacrylamide gel electrophoresis (Fig. 1). The iodinated preparations were routinely analyzed for structural homogeneity by NaDodSO4-polyacrylamide gel electrophoresis (Fig. 1). The iodination of basic FGF was 20% and that of acidic FGF was 25%. The specific activity of acidic FGF was 9 × 10^4 cpm/ng, whereas that of basic FGF was 2 × 10^4 cpm/ng. Both basic and acidic FGF were stable for 1 month after iodination. The content of free I25I in the final preparation was less than 0.2% as assessed by trichloroacetic acid precipitation. The biological activity of basic and acidic FGF assessed using adrenal cortex cells as target cells (12) was equal or higher than that of native basic or acidic FGF from the same batch.

Binding of Acidic and Basic FGF to BHK-21 Cells—BHK-21 cells were grown to confluency in the presence of DF medium supplemented with 5% calf serum in 16-mm wells (5–6 × 10^5 cells/well). Confluent cultures were transferred to 4 °C, and all subsequent operations were done in the cold. The cells were washed once with 500 μl of cold PBS, and 200 μl of a binding buffer composed of F-12 medium, 25 mM HEPES, pH 7.5, and 0.2% gelatin were added to each of the wells. Acidic or basic FGF was then added to the wells to the desired concentrations. Nonspecific binding was usually determined in the presence of an excess (2 μg) of pure unlabeled acidic or basic FGF. Both total and nonspecific binding were determined in duplicate wells. The cells were incubated for 4 h in the cold. At the end of the incubation, they were washed three times with 0.5 ml of PBS supplemented with 0.1% BSA. Subsequently, each well received 0.4 ml of 0.1% BSA and of X-100 in water, and 0.1% BSA. After 1 min, an aliquot was taken and the radioactivity was determined in a Beckman Model 5500 γ-counter. Nonspecific binding to the cells was usually about 20% or less of the total binding. At saturating concentrations of acidic or basic FGF, the nonspecific adsorption of 125I-FGF to the dishes did not exceed 5% of the total binding. All the experimental measurements were run in duplicate or triplicate, and all experiments were done at least three times.

Cross-linking of Basic or Acidic FGF to BHK-21 Cells—Baby hamster kidney cells were grown to confluence in 5-cm dishes. The dishes were washed twice with 10 ml of cold PBS, and 1.2 ml of binding buffer were then added to each dish. Basic and acidic FGF together or not with increasing concentrations of either native basic or acidic FGF were added to each dish to the final concentrations indicated in figure legends. The dishes were then incubated at 4 °C on a rotating platform at two cycles per minute. After the first 1 min of incubation, each dish was washed twice with 10 ml of cold PBS. DSS freshly dissolved in dimethyl sulfoxide to a concentration of 20 mM was diluted into PBS at room temperature to a final concentration of 0.15 mM (12). Two ml of this solution were added to each dish. The dishes were incubated at room temperature for 15 min. The reaction was then terminated by the addition of 200 μl of quenching buffer containing 10 mM Tris-HCl, pH 7.5, 200 mM glycine, and 2 mM EDTA. Each dish was incubated for 1 min at room temperature, and the dishes were subsequently put on ice. The incubation medium was aspirated, each dish was washed twice with 10 ml of cold PBS, and subsequently 0.7 ml of cold PBS containing 0.1 mM phenylmethylsulfonyl fluoride and 1 mM EDTA were added to each dish (12). The cells were scraped off with a rubber policeman, and the cell suspension was centrifuged for 30 s in a microcentrifuge. The supernatant was aspirated, and the pellet was solubilized in cold 1% sodium dodecyl sulfate (20 mM Tris-HCl, pH 7, 0.5% Nonidet P-40, 0.1% EDTA, and 1 mM phenylmethylsulfonyl fluoride) for 10 min. The suspensions were boiled for 5 min and electrophoresed in the discontinuous system of Laemmli (13) with 6 or 15% NaDodSO4-polyacrylamide gels (1.5 mm thick) with a 3% stacking gel. Samples were run reduced. The gels were fixed and stained with 0.1% Coomasie Blue in 50% trichloroacetic acid for 15 min and destained overnight with 7% acetic acid. The gels were then dried and subjected to autoradiography at −70 °C using Kodak X-Omat R film and DuPont Lightning Plus intensifying screens. High and low molecular weight protein markers were used to determine the molecular weight of the labeled bands.

Densityometry—Denstometric analysis of autoradiograms was performed using a Biomed Instruments model SL-504 Zeineh soft laser scanning densitometer. Autoradiograms were scanned, and the approximate area under each of the resulting peaks was evaluated by cutting it out of the paper and weighing. The area of the M, 140,000 peak was usually divided with the area of the M, 160,000 peak to give the ratio between areas under the peaks.

RESULTS

Comparison of the Biological Activity of Basic and Acidic FGF Using BHK-21 Cells and the Binding Characteristics of Acidic FGF to BHK-21 Cells—The effect of increasing...
concentrations of both basic and acidic FGF on the proliferation of BHK-21 cells exposed to DF medium supplemented with constant amounts of transferrin (1 μg/ml) and HDL (250 μg of protein/ml) is shown in Fig. 2. Basic FGF has a detectable effect when added at concentrations as low as 10 pg/ml and is saturating at 5 ng/ml. Its ED₅₀ is 500 pg/ml. Acidic FGF is 20-fold less potent than basic FGF (saturation at 100 ng/ml, ED₅₀ at 9 ng/ml), and its log dose response parallels that of basic FGF.

In order to minimize possible internalization, the binding of acidic ¹²⁵I-FGF to BHK-21 cells was analyzed at 4°C. As shown in Fig. 3, when binding is analyzed as a function of acidic ¹²⁵I-FGF concentration, apparent saturation of binding is achieved at 30 ng/ml. Nonspecific binding averaged 18–20%. At 4°C, the association reaches a plateau after 2 h, and the cell-associated radioactivity is fully displaced by excess native basic or acidic FGF, but not by excess EGF, insulin, or transferrin (data not shown). Scatchard analysis of the binding of acidic FGF as a function of concentration is shown in the inset of Fig. 3. A straight line was obtained by fitting the data using a linear least square program. The Scatchard plot indicates that a single class of binding sites is most likely over the concentration range of acidic FGF studied, and the apparent dissociation constant for the interaction of acidic ¹²⁵I-FGF and BHK-21 cells is 2.5 × 10⁻¹⁰ M. Approximately 87,000 binding sites are found per cell. This compares with an apparent dissociation constant for basic FGF of 2.7 × 10⁻¹⁰ M and 120,000 binding sites/cell (12).

Displacement of Basic or Acidic ¹²⁵I-FGF from BHK-21 Cell Membranes by Native Basic or Acidic FGF—To test whether acidic FGF can compete with basic FGF for similar binding sites, the ability of increasing concentrations of native basic and acidic FGF to displace basic versus acidic ¹²⁵I-FGF bound to a crude BHK-21 cell membrane preparation was analyzed. As shown in Fig. 4, both native basic and acidic FGF displace bound basic or acidic ¹²⁵I-FGF. The concentration of native basic FGF (10 ng/ml) required to displace 50% of bound basic ¹²⁵I-FGF was close to that of basic ¹²⁵I-FGF (14 ng/ml) present in the binding medium. In contrast, a 10-fold higher concentration of native acidic FGF (101 ng/ml) was required in order to observe a similar displacement. When the displacement of acidic ¹²⁵I-FGF by native basic and acidic FGF was analyzed, the concentration of native acidic FGF (11.5 ng/ml) required in the absence of competing FGF, 5 ng basic FGF indicated. The binding was performed as described under “Experimental Procedures.” When the binding was done in the absence of competing FGF, 5 ng basic (A) and 5.3 ng acidic (B) ¹²⁵I-FGF were bound to 1 mg of membrane protein (100%). Data are the average of duplicate determinations. The nonspecific absorption of ¹²⁵I-FGF to the filters was determined as described in Ref. 12. This value was subtracted from the results shown in the figure.
to displace 50% of bound acidic 125I-FGF was close to that of acidic 125I-FGF present in the binding medium (13 ng/ml). In contrast, a lower concentration of native basic FGF (5 ng/ml) was required for similar displacement. This suggests that both basic and acidic FGF can compete for similar binding sites. It should be noted that the shape of the competition curves obtained with native basic FGF in both experiments differs with respect to the curves obtained using native acidic FGF as the competing agent, suggesting that the interaction of basic and acidic FGF with their receptors may somehow differ.

Affinity Cross-linking of Basic and Acidic 125I-FGF to Their Respective Receptors—The membrane binding sites for basic and acidic FGF in intact BHK-21 cells were further characterized by affinity labeling. All experiments were done at 4 °C in order to label cell surface components and to limit possible internalization of growth factor receptor complexes.

When intact BHK-21 cells were incubated with 31 ng/ml basic or acidic 125I-FGF and then subjected to cross-linking with 0.15 mM DSS, the labeling of two major bands was observed. These bands migrated on electrophoresis in NaDodSO4-polyacrylamide gels under reducing conditions at positions corresponding approximately to M, 140,000 and 140,000, respectively, as visualized by autoradiography of the dried fixed gels (Fig. 5A, lane 1, and B, lane 2). Assuming that these weights include those of the bound FGF, the apparent molecular weights of the two labeled macromolecular species prior to cross-linking would be at the most M, 145,000 and 125,000. The apparent molecular weights of the two labeled species did not decrease when reducing agent was omitted prior to cross-linking.

A 1 2 3 4 5 6 7 8
160
B 1 2 3 4 5 6 7 8
160

FIG. 5. Affinity labeling of FGF receptors in BHK-21 cells by decreasing concentrations of basic or acidic 125I-FGF. Cultures of BHK-21 cells (5 × 10⁶ cells/5-cm culture dish) were incubated for 3 h at 4 °C with decreasing concentrations of basic 125I-FGF (A: lane 1, 31 ng/ml; lane 2, 21 ng/ml; lane 3, 10 ng/ml; lane 4, 5 ng/ml; lane 5, 2 ng/ml; lane 6, 1 ng/ml; lane 7, 0.35 ng/ml; lane 8, 0.17 ng/ml) or decreasing concentrations of acidic 125I-FGF (B: lane 1, 47 ng/ml; lane 2, 31 ng/ml; lane 3, 25 ng/ml; lane 4, 12 ng/ml; lane 5, 6 ng/ml; lane 6, 3 ng/ml; lane 7, 1 ng/ml; lane 8, 0.3 ng/ml). Cultures were incubated at 4 °C for 2.5 h. They were then washed and cross-linked to bound 125I-FGF with 0.15 mM DSS. After cross-linking, cells were scraped off the dishes, sedimented, and extracted with 0.5% Nonidet P-40. Aliquots were subjected to NaDodSO4-polyacrylamide gel electrophoresis on 1.5-mm thick gels. The molecular weight markers used were myosin (M, 200,000), a-galactosidase (M, 116,000), phosphorylase b (M, 92,000), BSA (M, 66,000), ovalbumin (M, 45,000), and carbonic anhydrase (M, 31,000). A similar migration pattern was observed regardless of whether or not samples were run under reducing conditions.

The intensity of labeling of the two labeled species as a function of decreasing concentrations of basic 125I-FGF (ranging from 31 to 0.35 ng/ml) and acidic 125I-FGF (ranging from 47 to 1 ng/ml) was analyzed by subjecting the autoradiograms to densitometric analysis (Fig. 6). In the case of basic 125I-FGF at concentrations of 10 ng/ml (Fig. 5A, lane 3), the ratio between the intensity of the M, 140,000 and 160,000 complexes was 1.5 (Fig. 6, upper). As the concentration of basic 125I-FGF decreased, the ratio between the M, 140,000 and 160,000 complexes decreased steadily, reaching a value of 0.39 (Fig. 6, upper) at a concentration of basic 125I-FGF of 0.35 ng/ml (Fig. 5A, lane 7). This can be interpreted as indicating that basic 125I-FGF has a higher affinity for the M, 145,000 receptor species.

In the case of acidic 125I-FGF at a concentration of 25 ng/ml (Fig. 5B, lane 3), the ratio between the intensity of the M, 140,000 and 160,000 complexes was 3.15 (Fig. 6, lower), therefore being higher than that observed with basic 125I-FGF. Also, in contrast with basic 125I-FGF, the ratio between the M, 140,000 and 160,000 complexes did not change much as the concentration of 125I-FGF decreased from 47 to 1 ng/ml (Figs. 5B and 6, lower) and reached a value of 2.4 at the lowest acidic 125I-FGF concentration (Fig. 5B, lane 7, and Fig. 6, lower). Thus, it appears that acidic 125I-FGF has about the same affinity for both macromolecular species.

Effect of Increasing Concentrations of Native Basic Versus Acidic FGF on the Cross-linking of Basic and Acidic 125I-FGF to BHK-21 Cells—In order to establish whether or not the M, 140,000 and 160,000 complexes radiolabeled with basic 125I-FGF were similar to those observed with acidic 125I-FGF, BHK-21 cells were exposed to basic 125I-FGF (14 ng/ml) in the absence or presence of increasing concentrations of native basic FGF ranging from 15 to 400 ng/ml (Fig. 7A) or acidic FGF ranging from 50 to 2400 ng/ml (Fig. 7B). After cross-linking, the distribution of 125I-FGF between the M, 140,000 (data not shown), suggesting that neither of the two species was composed of disulfide-linked subunits (12).
and 160,000 complexes was analyzed by NaDodSO4-polyacrylamide gel electrophoresis followed by autoradiography and densitometric analysis of the resulting autoradiograms. When basic 125I-FGF was cross-linked in the absence of an excess of either native basic or acidic FGF, the ratio as determined by densitometry between the intensity of the M, 140,000 and 160,000 complexes was 1.67 (Fig. 7A, lane 1). When the cross-linking was done in the presence of increasing concentrations of native basic FGF, both complexes showed a more or less parallel decrease in their labeling intensity, and the ratio between their intensity remained 1.61 even when the cross-linking was done in the presence of 400 ng/ml native basic FGF (Fig. 7A, lane 5). In contrast, when the cross-linking of basic 125I-FGF was done in the presence of increasing concentrations of native acidic FGF, a preferential decrease in the labeling of the M, 140,000 complexes was observed, so that when labeling was done in the presence of 50 ng/ml native acidic FGF (Fig. 7B, lane 2), its ratio became 0.6, and at 2.4 ng/ml of native acidic FGF, the ratio became 0.28, reflecting the almost total quenching of the labeling intensity of the M, 140,000 complexes as compared to the M, 160,000 complexes. It was only at concentrations above 400 ng/ml native acidic FGF that appreciable decreases in the labeling intensity of the M, 160,000 complexes were observed (Fig. 7B). These observations tend to indicate that native acidic FGF can compete with basic 125I-FGF for binding to both the M, 125,000 and 145,000 receptor species and appears to displace basic 125I-FGF more efficiently from the M, 125,000 species than from the M, 145,000 species. Therefore, acidic FGF appears to possess a higher affinity than basic 125I-FGF for the M, 125,000 receptor species.

To further demonstrate the different affinities of acidic versus basic FGF for the two macromolecular species, cultures were exposed to acidic 125I-FGF in the absence or presence of increasing concentrations of native acidic FGF ranging from 23 to 180 ng/ml (Fig. 8A) or native basic FGF ranging from 20 to 640 ng/ml (Fig. 8, A and C). After cross-linking, the distribution of acidic 125I-FGF between both macromolecular species was analyzed by NaDodSO4-polyacrylamide gel electrophoresis, followed by autoradiography and densitometry. When the cross-linking of acidic 125I-FGF was done in the presence of increasing concentrations of native acidic FGF, both the M, 160,000 and 140,000 complexes showed a marked parallel decrease in their labeling intensity which was nearly quenched when the labeling was done in the presence of 90 ng/ml native acidic FGF (Fig. 8A, lane 6). The ratio between the intensity of the M, 140,000 and 160,000 complexes, which was 2.0 in the absence of competing native acidic FGF, remained 2.0, although the absolute intensity of each of the labeled bands was greatly diminished when the cross-linking was done in the presence of 81 ng/ml native acidic FGF (Fig. 8, lanes 1, 5, 6, and 7). Labeling of both macromolecular species by acidic 125I-FGF was specific, since it could not be displaced by EGF (2 μg/ml), insulin (5 μg/ml), or transferrin (5 μg/ml), whereas it was totally quenched by basic FGF (2 μg/ml) (Fig. 8B). When cells were exposed to acidic 125I-FGF in the presence of increasing concentrations of native basic FGF at concentrations as low as 20 ng/ml native basic FGF appeared to totally quench the labeling of the M, 160,000 complex (Fig. 8C, lane 2). It was not, however, until concentrations as high as 320 ng/ml native basic FGF were reached that the labeling of the M, 140,000 complex was almost totally quenched (Fig. 8C, lane 6). These results therefore confirm that basic FGF has a higher affinity than acidic FGF for the M, 145,000 receptor species, whereas acidic FGF displays a higher affinity than basic FGF for the M, 125,000 receptor species.

**DISCUSSION**

The BHK-21 cell line has been proposed as a model for the study of the interaction of basic FGF with cell surface receptors, since those cells require a 10-20-fold higher basic FGF concentration than other normal diploid cells in order to proliferate actively (7, 8, 10). Previous studies (12) have demonstrated the presence of high affinity basic FGF receptors in BHK-21 cells (KD = 2.7 × 10−10 M, 1.2 × 105 receptors/cell). As with other cell types (7, 8, 10), BHK-21 cells also respond to acidic FGF, and concentrations 20-fold higher than those of basic FGF are required in order to stimulate cells to proliferate to the same extent. As in the case of basic FGF, BHK-21 cells have high affinity receptors for acidic FGF (KD = 2.5 × 10−10 M, 87,000 receptors/cell). Since others have shown that addition of platelet-derived growth factor (14), basic FGF (15), or β-transforming growth factor (16) to intact cells could affect the expression of other growth factor receptors, such as that of the EGF receptor, we have chosen to use crude membrane preparation rather than intact cells to study whether basic and acidic FGF could compete for similar cell surface receptors. In a cell membrane preparation, displacement of one type of FGF by the other should reflect a true competition at the receptor level, rather than modulation of receptor number or affinity. Since acidic FGF can displace membrane-bound basic 125I-FGF, it suggests that acidic FGF competes for basic FGF receptors. This is supported by the results of the reverse experiment where displacement of acidic 125I-FGF by native basic FGF was observed. This suggests that basic FGF competes for acidic 125I-FGF.
receptor sites. Since the shape of the competition curve with acidic FGF differs from that of basic FGF, it indicates a difference in the mode of competition of the two mitogens for the receptor sites.

In order to unambiguously establish that basic and acidic FGF interact with the same receptor species, we have used a technique that was developed to cross-link membrane-bound 125I-labeled ligands to membrane components and has been used to affinity-label and characterize receptors specific for insulin (17, 18), insulin-like growth factors (19, 20), EGF (21), α- and β-transforming growth factors (21–24), platelet-derived growth factor (25), and nerve growth factor (26).

Our results show that basic as well as acidic 125I-FGF bound to BHK-12 cells become covalently attached to two macromolecular components when DSS is used as the cross-linking agent. That the Mr 160,000 and 140,000 species from BHK-21 cells represent the cross-linked basic or acidic 125I-FGF receptors is suggested by 1) the labeling of these two species occurred at biologically relevant concentrations of basic or acidic 125I-FGF and increasing concentrations of native basic FGF (A: lane 2, 27 ng/ml; lane 3, 81 ng/ml; lane 4, 162 ng/ml), native acidic FGF (A: lane 5, 25 ng/ml; lane 6, 91 ng/ml; lane 7, 180 ng/ml), 2 µg/ml EGF (B, lane 2), 5 µg/ml insulin (B, lane 3), 5 µg/ml transferrin (B, lane 4), 2 µl/ml acidic FGF (B, lane 5), or increasing concentrations of basic FGF (C: lane 2, 20 ng/ml; lane 3, 40 ng/ml; lane 4, 80 ng/ml; lane 5, 160 ng/ml; lane 6, 320 ng/ml; lane 7, 640 ng/ml). Cells were also incubated with 580 ng/ml acidic FGF (C, lane 8) or 16 ng/ml basic 125I FGF (C, lane 9). At the end of the incubation, the cells were washed, affinity-labeled, and extracted in the presence of 0.5% Nonidet P-40 as described under "Experimental Procedures." The extracts were reduced and subjected to NaDodSO4-polyacrylamide gel electrophoresis on 1.5-mm thick 6% gels. Shown is an autoradiogram (2-day exposure) from the resulting fixed, dried gel. The protein size markers were as described in the legend to Fig. 5.

**Fig. 8.** Affinity labeling of FGF receptors in BHK-21 cells by acidic 125I-FGF in the absence or presence of increasing concentrations of native acidic or basic FGF; lack of displacement of acidic 125I-FGF by EGF, insulin, or transferrin. Confluent BHK-21 cells were incubated for 3 h at 4 °C with 25 ng/ml acidic 125I-FGF alone (A–C, lane 1), 25 ng/ml acidic 125I-FGF and increasing concentrations of native basic FGF (A: lane 2, 27 ng/ml; lane 3, 81 ng/ml; lane 4, 162 ng/ml), native acidic FGF (A: lane 5, 25 ng/ml; lane 6, 91 ng/ml; lane 7, 180 ng/ml), 2 µg/ml EGF (B, lane 2), 5 µg/ml insulin (B, lane 3), 5 µg/ml transferrin (B, lane 4), 2 µl/ml acidic FGF (B, lane 5), or increasing concentrations of basic FGF (C: lane 2, 20 ng/ml; lane 3, 40 ng/ml; lane 4, 80 ng/ml; lane 5, 160 ng/ml; lane 6, 320 ng/ml; lane 7, 640 ng/ml). Cells were also incubated with 580 ng/ml acidic FGF (C, lane 8) or 16 ng/ml basic 125I FGF (C, lane 9). At the end of the incubation, the cells were washed, affinity-labeled, and extracted in the presence of 0.5% Nonidet P-40 as described under "Experimental Procedures." The extracts were reduced and subjected to NaDodSO4-polyacrylamide gel electrophoresis on 1.5-mm thick 6% gels. Shown is an autoradiogram (2-day exposure) from the resulting fixed, dried gel. The protein size markers were as described in the legend to Fig. 5.
acidic 125I-FGF from its cell surface binding sites. Those experiments show clearly that basic FGF displaces acidic 125I-FGF preferentially from the $M_1$, 145,000 receptor species and, to a lesser degree, from the $M_2$, 125,000 receptor species.

The relationship between the two receptor species can be interpreted in a number of ways. 1) It is possible that the lower $M_1$ receptor species is a proteolytic breakdown product of the larger species. Such a proteolytic event could occur before or during solubilization of the cells. If the lower molecular weight complexes represent an inactive proteolytic digestion product of the FGF receptor, then the higher affinities displayed by basic and acidic FGF toward the $M_1$, 145,000 receptor species seem to account for their differences in biological activities. 2) The $M_1$, 125,000 receptor species could represent a translational product of a truncated form of the mRNA coding for $M_1$, 145,000 receptor species. 3) The two complexes are structurally unrelated and are involved in different biological functions of FGF. Studies are being conducted at present in order to clarify the relationship between the two labeled receptor species.

Although the present study was limited to BHK-21 cells, which express a large number of putative FGF receptors, similar experiments done with normal diploid cells (adrenal cortex-derived capillary and human umbilical endothelial cells) have revealed the existence of similar cell surface components to which basic and acidic FGF can be cross-linked.

In conclusion, the present results establish the molecular basis for the similar biological activity of basic and acidic FGF. Qualitatively, both mitogens interact with the same membrane components ($M_1$, 145,000 and 125,000 species). Quantitatively, basic FGF appears to display a higher affinity than acidic FGF for the $M_1$, 145,000 receptor species, whereas acidic FGF displays a higher affinity than basic FGF for the $M_1$, 125,000 receptor species. In a recent study (27) comparing the structures of acidic and basic FGF, a 53% (75 out of 141 amino acids) absolute homology was found to exist between these mitogens. Up to 41 of the remaining 65 residues (64%) may involve nucleotide substitutions where a single base change could result in amino acid replacement and concomitant homology. Such highly homologous structures suggest that acidic and basic FGF possess similar mechanisms of action and will interact with their cell surface receptor through similar binding domains localized in regions where the degree of homology between basic and acidic FGF is the highest.

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