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Primary structure of bovine pituitary basic fibroblast growth factor (FGF) and comparison with the amino-terminal sequence of bovine brain acidic FGF

(endothelial cell growth factor/angiogenesis/microsequencing/heparin)

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ABSTRACT **The two major mitogenic polypeptides for endothelial cells have been purified to homogeneity. The complete primary structure of bovine pituitary basic fibroblast growth factor (FGF) and the amino-terminal amino acid sequence of bovine brain acidic FGF have been established by gas-phase sequence analyses. Homogeneous preparations of these polypeptides are potent mitogens (basic FGF, $ED_{50} \approx 60$ pg/ml; acidic FGF $ED_{50} \approx 6000$ pg/ml) for many diverse cell types including capillary endothelial cells, vascular smooth muscle cells, and adrenocortical and granulosa cells; *in vivo*, basic FGF is a powerful angiogenic agent in the chick chorioallantoic membrane assay. The available protein sequence data demonstrate the existence of significant structural homology between the two polypeptides.**

The existence of endothelial cell growth factors has been demonstrated in a wide variety of tissue sources including bovine cartilage (1), hypothalamus (2–6), pituitary (7), brain (8, 9), retina (3, 10), macrophages (11), corpus luteum (12), adrenal gland (13), a rat chondrosarcoma (14), and human brain (15). The unusual heparin-binding ability of these mitogens (14) has provided an extremely powerful tool for the purification of these proteins, but none have yet been completely characterized structurally. Preliminary physicochemical analyses of some of these mitogens by $NaDodSO_4$/PAGE, isoelectric focusing, and assessment of their respective binding affinities for heparin have suggested that two general structures may be present; these have been referred to as the acidic (acidic FGF) and basic (basic FGF) fibroblast growth factors. *In vitro*, both molecules stimulate the proliferation of multiple endothelial cell types (16, 17) and, *in vivo*, basic FGF has been shown to be a potent angiogenic agent (18). The isolation and amino-terminal amino acid sequences of bovine basic FGF from multiple tissues (7, 12, 13, 19) and of acidic FGF from bovine brain (17) have been reported. Here we report the complete primary structure of bovine pituitary basic FGF and compare it to the amino-terminal amino acid sequence of bovine brain acidic FGF.

MATERIALS AND METHODS

Radioimmunoassay (RIA) for Basic FGF. Antibodies were generated against a synthetic analog of the amino-terminal sequence of basic FGF, [Tyr^{10}]FGF-(1–10) (conjugated to bovine serum albumin) and were subsequently used to develop the RIA for basic FGF (20).

Bioassays. Mitogenic activity of purified basic and acidic FGF was determined using cultures of bovine brain- and adrenal cortex-derived capillary endothelial cells, human umbilical vein endothelial (HUE) cells, bovine adrenal cortex cells, granulosa cells, and vascular smooth muscle cells. Culture and assay conditions were similar to those described earlier (16, 21, 22). Brain- and adrenal cortex-derived capillary endothelial cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum, and HUE cells were maintained in medium 199 supplemented with 20% fetal calf serum. Adrenal cortex cells were maintained in Ham's F-12 medium supplemented with 10% calf serum, granulosa cells were maintained in DMEM supplemented with 1% calf serum, and vascular smooth muscle cells were maintained in DMEM supplemented with 5% defibrinated bovine plasma. All cells were seeded at an initial density of 2×10^4 cells per 35-mm dish, except for HUE cells, which were seeded at 4×10^4 per 35-mm dish. Angiogenic assays were performed using the chorioallantoic membrane of chicken embryo as described (12).

Isolation of Basic FGF. Frozen bovine pituitaries were homogenized with a Waring blender for 5 min in 0.15 M ammonium sulfate (4 liters/kg of tissue). The pH was then adjusted to 4.5 with HCl and the homogenate was stirred vigorously for 2 hr. After centrifugation (18,000 × *g*, 30 min), 230 g of ammonium sulfate per liter of supernatant was added, the pH was adjusted to 6–6.5 with NaOH, and precipitation was allowed to proceed for ≥15 hr. After centrifugation (18,000 × *g*, 30 min), 300 g of ammonium sulfate was added per liter of supernatant and the mixture was stirred well for 2 hr. After centrifugation (18,000 × *g*, 30 min), the pellet from 3 kg of starting tissue was dissolved in 200 ml of distilled water and dialyzed against 20 liters of distilled water overnight. The pH of the dialyzed retentate was then adjusted to 6 and the solution was clarified by centrifugation (12,000 × *g*, 30 min). Basic FGF was subsequently isolated by three different protocols; two of these employed conventional ion-exchange and reversed-phase HPLC purification steps as described (7, 16), whereas the third method utilized heparin-Sepharose affinity chromatography as described by Gospodarowicz *et al.* (9) in a key purification step as detailed in Fig. 1. Bovine brain acidic FGF was isolated with a protocol similar to that depicted in Fig. 1 (17).

Modification of Cysteine Residues. Cysteine residues were either reduced and alkylated with iodo[^{14}C]acetamide (New England Nuclear) or oxidized with performic acid as indicated below. In either case, the FGF in 0.1% trifluoroacetic acid/acetonitrile was dried in a 1.5-ml polypropylene microcentrifuge tube in a Speed Vac vacuum centrifuge (Savant) just prior to modification.

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Abbreviations: FGF, fibroblast growth factor; RPLC, reversed-phase liquid chromatography.

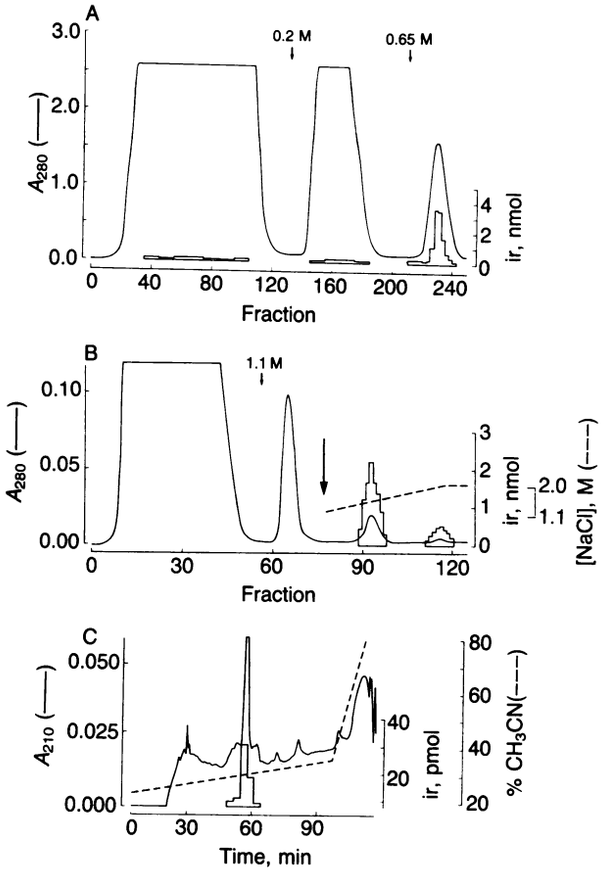

FIG. 1. Purification of bovine pituitary basic FGF. (*A*) CM-Sephadex C-50 ion-exchange chromatography. A 7 × 9 cm column was washed with 1 liter of 50 mM sodium phosphate, pH 6.0/1.5 M NaCl and then equilibrated with 0.1 M sodium phosphate (pH 6.0). The crude extract from 3 kg of bovine pituitaries was loaded onto the column, and the column was washed sequentially with 0.1 M sodium phosphate (pH 6.0) containing no NaCl, 0.2 M NaCl, and 0.65 M NaCl; the A_{280} was allowed to reach a minimum before initiation of each new wash. Fractions (18 ml) were collected at 3 ml/min at 4°C and subjected to RIA. ir, [Tyr^{10}]FGF-(1–10) immunoreactive equivalents. (*B*) Heparin-Sepharose chromatography. The 0.65 M NaCl eluate from CM-Sephadex chromatography was loaded onto a 3 × 3 cm column of heparin-Sepharose (Pharmacia) previously equilibrated with 10 mM Tris Ci, pH 7.0/0.6 M NaCl at room temperature. The column was then washed sequentially with 10 mM Tris Cl (pH 7.0) containing 0.6 M NaCl and 1.1 M NaCl; the A_{280} was allowed to reach a minimum with each wash. The basic FGF then (long arrow) was eluted with a 200-ml linear 1.1–2 M NaCl gradient in 10 mM Tris Cl (pH 7.0). Fractions (5 ml) were collected at 0.8 ml/min and subjected to RIA. (*C*) RPLC. The basic FGF from heparin-Sepharose chromatography was pumped onto a Vydac C_4 (0.46 × 25 cm) reversed-phase column (The Separations Group, Hesperia, CA), using a 0.1% trifluoroacetic acid/acetonitrile solvent system, and eluted at 0.6 ml/min with a 90-min gradient of 23–35% acetonitrile. Fractions (1.8 ml) were collected at room temperature and subjected to RIA. A pilot run with 0.1% of the heparin-Sepharose-purified basic FGF is shown.

Reduction and alkylation. The dried FGF was dissolved in 0.1 ml of deoxygenated 0.5 M Tris Cl, pH 7.7/10 mM EDTA/6 M guanidinium chloride. Dithiothreitol was added to a final concentration of 5–10 mM and reduction was allowed to proceed at 37°C for 30 min. A 0.5-fold molar excess of iodo[^{14}C]acetamide (24 mCi/mmol; 1 Ci = 37 GBq) over total sulfhydryl groups was added and the incubation was continued at 37°C for 60 min in the dark. The alkylation was terminated by addition of a large excess of dithiothreitol over iodoacetamide and the alkylated FGF was purified by reversed-phase liquid chromatography (RPLC).

Table 1. Amino acid compositions of basic FGF from different bovine tissues

Amino acid	mol of amino acid/mol of protein			
	Brain*	Hypothalamus*	Pituitary	Basic FGF-(1–146)†
Asx	13.7	13.0	12.4 ± 0.4	12
Thr	5.1	4.9	3.9 ± 0.3	4
Ser	10.0	10.0	9.4 ± 0.6	10
Glx	13.2	14.2	14.1 ± 0.4‡	12
Pro	11.6	11.3	9.4 ± 0.6	10
Gly	17.3	18.2	16.6 ± 0.6‡	15
Ala	9.1	9.0	9.5 ± 0.4	9
Cys	ND	ND	4.3 ± 0.2§	4
Val	5.8	5.7	5.9 ± 0.7	7
Met	2.4	2.4	1.6 ± 0.4	2
Ile	3.2	3.1	3.4 ± 0.5	4
Leu	12.6	12.9	13.4 ± 0.4	13
Tyr	6.5	6.2	6.8 ± 0.4	7
Phe	7.9	7.6	7.5 ± 0.2	8
His	3.2	3.2	2.4 ± 0.6	3
Lys	13.7	13.5	13.9 ± 0.7	14
Arg	10.8	10.4	11.6 ± 0.3	11
Trp	ND	ND	0.4 ± 0.2	1

ND, not determined.
*Data from Lobb and Fett (6) normalized for 146 amino acids.
†Amino acid composition of basic FGF deduced from sequence analysis.
‡Discrepancy between amino acid and sequence analysis data is greater than that expected from statistical analysis.
§Cysteine was determined as cysteic acid after RPLC purification of performic acid-oxidized basic FGF.

Performic acid oxidation. Performic acid was generated by incubating 9 ml of distilled formic acid with 1 ml of 30% H_2O_2 at room temperature in a tightly capped tube for 1 hr. Dried FGF (5–15 nmol) was dissolved in 0.25 ml of the resulting solution, and oxidation was permitted to continue at 0°C for 2.5 hr. Four lyophilizations from distilled water were employed to remove reaction byproducts.

Proteolytic and Chemical Digestions of Basic FGF. Prior to any digestion the FGF (with or without modified cysteines) was dried in a polypropylene microfuge tube in a Speed Vac vacuum centrifuge to remove volatile RPLC solvents.

Proteolytic digestions. The dried FGF (1–5 nmol) was dissolved in 0.01 ml of 0.5 M Tris Cl, pH 7.7/10 mM EDTA/6 M guanidinium Cl and then diluted to 1 ml with 1% NH_4HCO_3. Submaxillaris protease or chymotrypsin was added in a 1:50 (wt/wt) ratio, whereas digestions with *Staphylococcus aureus* V8 protease employed a 1:35 (mol/mol) ratio of enzyme to substrate. Incubations were allowed to proceed overnight at 37°C.

Cyanogen bromide digestions. The dried, alkylated FGF (5–6 nmol) was dissolved with 0.05 ml of 70% formic acid and reduced in a solution of 2.9 M *N*-methylmercaptoacetamide in 7% formic acid (23) for 24 hr at 37°C. The alkylated, reduced FGF was purified by RPLC, dried in a Speed Vac vacuum centrifuge, and redissolved in 0.1 ml of deoxygenated 70% formic acid. A 100-fold excess of cyanogen bromide was added and the incubation was continued at room temperature in the dark overnight.

RPLC purifications of modified FGF and its digestion fragments were accomplished with a Brownlee RP-300 reversed-phase column (0.46 × 25 cm) and a 0.1% trifluoroacetic acid/acetonitrile or a 0.1% heptafluorobutyric acid/acetonitrile solvent system (24).

Structural Characterization. Amino acid analyses and gas-phase microsequencing of intact FGF and its digestion fragments were carried out by methods previously described (25, 26). The phenylthiohydantoin derivative of [^{14}C]carboxy-

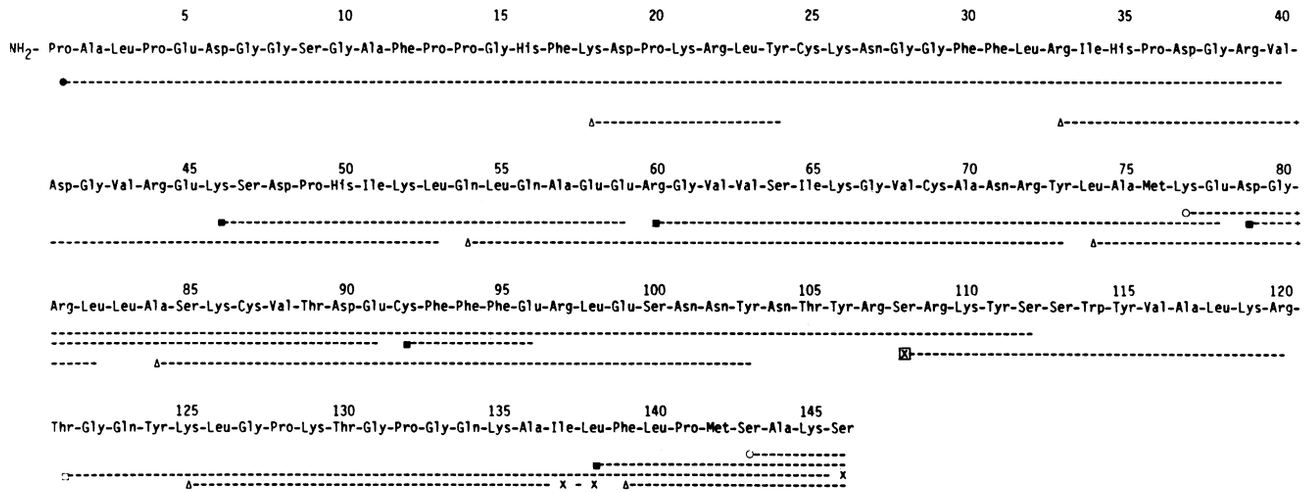

FIG. 2. Primary structure of bovine pituitary basic FGF. The sequence is shown with all peptides necessary for proving the structure. Sequence data obtained from intact FGF (●), cyanogen bromide peptides (○), *S. aureus* V8 peptides (■), submaxillaris protease peptides (□), and chymotryptic peptides (△) are indicated. Ambiguous amino acid assignments are denoted by x.

amidomethylcysteine was identified during sequence analysis by liquid scintillation counting of the residues from the sequencer. The identification of cysteic acid in a given cycle was accomplished by comparison of the amino acid composition of the peptide and the remainder of its sequence as determined by Edman degradation. Carboxypeptidase Y was obtained from Pierce and utilized according to the manufacturer's recommendations. Carboxyl-terminal analysis via tritium incorporation was accomplished as described (27).

RESULTS

Isolation of Bovine Pituitary Basic FGF. The highly efficient purification procedure described in Fig. 1 permitted the rapid isolation of large quantities (60 nmol per week) of basic FGF from 3 kg of bovine pituitaries (28). The heparin-Sepharose affinity-chromatography step resulted in a several thousandfold purification of two biologically active and basic FGF-immunoreactive mitogens, which were eluted at ≈1.4 M and ≈1.95 M NaCl (Fig. 1*B*). A single RPLC step effected peptide homogeneity in each case. $NaDodSO_4$/PAGE yielded identical molecular weight estimates for both species and gas-phase microsequencing showed that both possessed identical amino-terminal amino acid sequences through at least the amino-terminal 24 residues of each polypeptide (data not shown). Pituitary extracts yielded ≈15 times more of the mitogen eluted at 1.4 M NaCl than of the species eluted later; hence, the former was selected for further structural characterization.

Amino Acid Analyses and Microsequencing of Basic FGF. $NaDodSO_4$/PAGE suggested a molecular weight of 16,250 ± 1000 (data not shown) for bovine pituitary basic FGF, which is in close agreement with M_r 16,415 for the established 146 amino acid structure of basic FGF. Table 1 shows the amino acid compositions obtained for the cationic mitogen from bovine brain and hypothalamus by Lobb and Fett (6) as well as our compositional data for basic FGF from bovine pituitary, all data being normalized for a 146 amino acid structure. The similarity of the compositions suggests that these structures are closely related, if not identical. We have, in fact, isolated basic FGF from bovine brain (9) and determined that its amino-terminal sequence is identical to that of pituitary basic FGF (19).

Fig. 2 shows the primary structure of bovine pituitary basic FGF as derived from the sequence analyses of the intact molecule and some of its various digestion fragments. In total, more than 59 proteolytic and chemical-digestion fragments from basic FGF were characterized with the gas-phase sequencer, permitting the multiple confirmation of each amino acid assignment (remainder of data not shown in Fig. 2). The proteolytic generation of short, easily characterized peptide fragments allowed the unambiguous assignment of the entire basic FGF-(1–146) sequence. Supporting evidence for the accuracy of the amino acid sequence can be found in

Table 2. Amino-terminal sequence analysis of [^{14}C]carboxyamidomethylated bovine brain acidic FGF (750 pmol)

Residue	>PhNCS-AA*	Yield, pmol
1	Phe	461
2	Asn	324
3	Leu	276
4	Pro	185
5	Leu	274
6	Gly	190
7	Asn	192
8	Tyr	130
9	Lys	85.6
10	Lys	117
11	Pro	93.6
12	Lys	54.2
13	Leu	59.1
14	Leu	73.2
15	Tyr	73.5
16	Cys	18.7†
17	Ser	42.5
18	Asn	68.1
19	Gly	22.4
20	Gly	79.1
21	Tyr	26.6
22	Phe	34.7
23	Leu	33.9
24	Arg	24.3
25	Ile	19.3
26	Leu	26.9
27	Pro	24.3
28	Asp	0.25
29	Gly	14.4

Initial yield = 41.7%; average repetitive yield = 89.1%; average lag per cycle = 1.1%.
*Phenylthiohydantoin amino acid derivative.
†Quantitated by cpm released as >PhNCS-[^{14}C]carboxyamidomethylcysteine.

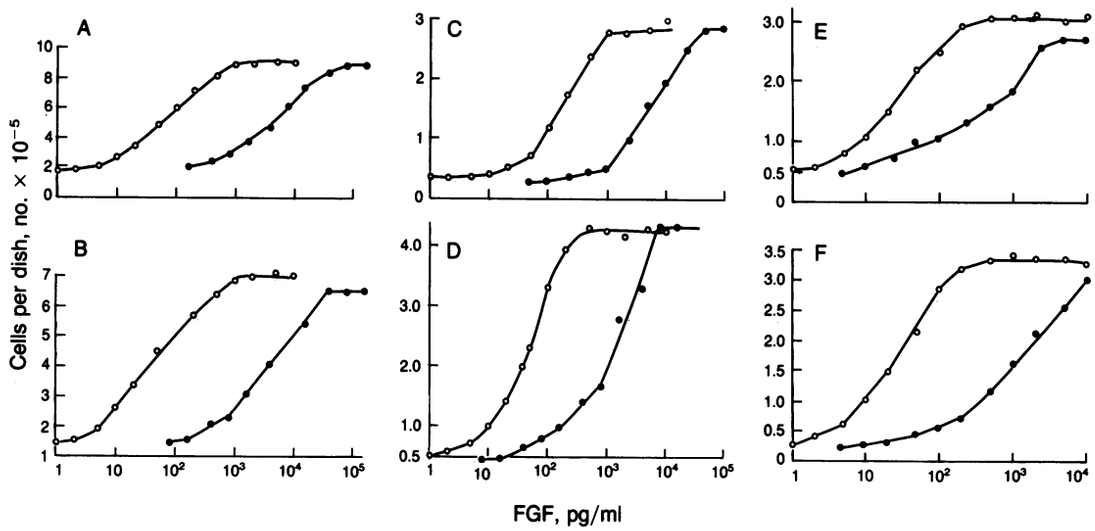

FIG. 3. Effects of acidic (●) and basic (○) FGF on the proliferation of various mesoderm-derived cell types *in vitro*. (*A*) Capillary endothelial cells from bovine adrenal cortex. (*B*) Capillary endothelial cells from bovine brain. (*C*) Human umbilical vein endothelial cells. (*D*) Bovine adrenal cortex cells. (*E*) Granulosa cells. (*F*) Vascular smooth muscle cells. Cells were seeded in triplicate at low density (2 or 4 × 10^4 cells per 35-mm dish) and maintained in the presence of various concentrations of either acidic (●) or basic (○) FGF (10 μl aliquots added on days 2 and 4) for 5–6 days, after which they were removed from the plates by use of trypsin and then were counted in a Coulter counter.

Table 1. The amino acid compositions of basic FGF from amino acid analyses and microsequencing data are almost identical, particularly when the statistical deviations of the amino acid-analyses results are considered. The two discrepancies (Glx and Gly) may be attributable to the obvious difficulty in obtaining perfect whole number ratios of relatively abundant amino acids in large polypeptides.

The cysteine content (4.3 mol of Cys/mol of FGF) of basic FGF(1–146) in Table 1 was determined after performic acid oxidation and RPLC purification of the protein. By amino acid analysis, two additional cysteine residues (i.e., 6.0 mol of Cys/mol of FGF) are seen without prior modification of cysteines, and performic acid oxidation alone (i.e., without HCl hydrolysis) generates 1.45 mol of Cys/mol of FGF. Hence, up to 2 mol of free cysteine may be bound per mol of basic FGF.

Carboxyl-Terminal Sequence Identification of Basic FGF. Absolute identification of the carboxyl-terminal residue of bovine pituitary basic FGF has not been established, as carboxypeptidase Y and carboxyl-terminal tritium-incorporation experiments have yielded no meaningful data. However, we have sequenced three basic-FGF peptide fragments from cyanogen bromide [FGF-(143–146)], *S. aureus* V8 [FGF-(138–146)], and chymotryptic [FGF-(139–146)] digestions (Fig. 2), and all terminate with serine. The specificity of these digestions, especially that of the cyanogen bromide reaction, strongly suggests that cleavage at Ser-146 of basic FGF is very unlikely and thus imply that Ser-146 is, in fact, the carboxyl-terminal amino acid of bovine pituitary basic FGF.

Isolation and Amino-Terminal Sequence Analysis of Bovine Brain Acidic FGF. Bovine brain acidic FGF was isolated as described (17), reduced with dithiothreitol, and alkylated with iodo[^{14}C]acetamide. The results of gas-phase sequence analyses of 750 pmol of intact [^{14}C]carboxyamidomethylated acidic FGF are shown in Table 2.

Biological Activities of Acidic and Basic FGFs. The mitogens sequenced in these studies appear to have all of the *in vitro* biological activities of the acidic and basic FGFs (Fig. 3). They are potent (acidic FGF, $ED_{50} \approx 6000$ pg/ml; basic FGF, $ED_{50} \approx 60$ pg/ml) stimulators of the proliferation of several distinct cell types, and basic FGF can induce neovascularization *in vivo* (Fig. 4).

DISCUSSION

A growing body of physicochemical evidence has suggested that acidic FGF and basic FGF are the two major mitogens responsible for stimulating endothelial-cell proliferation. Amino-terminal sequence analyses have shown (Table 3) that basic FGF can be isolated from a variety of tissues, including bovine brain (19), adrenal gland (13), corpus luteum (12), retina and kidney (unpublished observations) and human brain (15), and that acidic FGF can be isolated from bovine brain (17). The Gly^{15}-His^{16} bond of basic FGF is susceptible to cleavage (Table 3), which may explain why the purified protein is sometimes seen as a doublet on NaDodSO$_4$/PAGE

Table 3. Amino-terminal sequence analysis of bovine basic FGF isolated from various tissues

Tissue	Amount of FGF sequenced, pmol	Amino-terminal sequence*							Reference
		5	10	15	20	25	30	35	
Brain	200	PALPE	DGGSX	AFPPG	XFK				19
Adrenal gland†	210	PALPE	DXGXG	AFXP					13
Retina	500	PALPE	DGGSG	AFPPG	HFKDP	KRLYX	KNGGF	FLRIH	Unpublished
Corpus luteum‡	72	HFKDP	KRLYX	KNGGX	FLRIX	PD			12
Kidney‡	71	HFKDP	KRLYX	KNGXF	FLO				Unpublished

*The standard one-letter amino acid abbreviations are used.
†Adrenal gland preparations have also been shown to contain the FGF-(16→) fragment.
‡FGF-(16→) sequence.

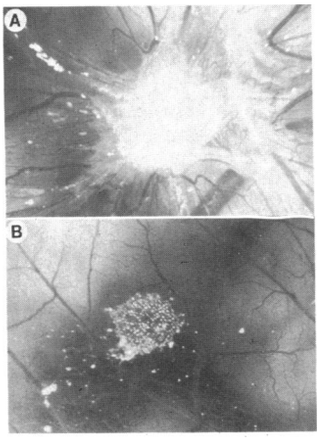

FIG. 4. Vascular response induced by Sephadex G-200 beads containing (*A*) or not containing (*B*) 500 ng of basic pituitary FGF. Beads were implanted onto the chorioallantoic membrane of 9-day-old chicken embryos and the zone of implantation was photographed 3 days later. (×40.)

(13). Whether this degradation is an artifact of the extraction process or results from tissue-specific processing of the protein is unknown.

There are several structural features of the basic FGF-(1–146) sequence that may bear on its mitogenic activity. (*i*) Pierschbacher and Ruoslahti (29, 30) have shown that the minimal cellular recognition site in the adhesion glycoprotein fibronectin is Arg-Gly-Asp-Ser and that conservative substitutions in the Ser position do not abrogate cell-attachment-promoting activity. Secondary structural analysis (31) of basic FGF-(1–146) predicts that basic FGF-(37–40) (Pro-Asp-Gly-Arg) and basic FGF-(78–81) (Glu-Asp-Gly-Arg) (exact inverses of the Arg-Gly-Asp-Xaa fibronectin sequence) may exist on the exterior of the protein and thus would be available for interactions with cellular surfaces in a potential cell-attachment-promoting activity. (*ii*) Based on amino acid sequence information from fibronectin, anti-thrombin III, and platelet factor 4, Schwarzbauer *et al.* (32) suggested that clusters of basic residues and pairs and groups of basic and aromatic residues may be involved in the binding of heparin to these proteins. Such sequences may be found in basic FGF-(18–22) (Lys-Asp-Pro-Lys-Arg) and basic FGF-(107–110) (Arg-Ser-Arg-Lys). (*iii*) A nearly perfect inverted repeat exists between basic FGF-(32–39) (**Leu**-Arg-**Ile**-**His**-**Pro**-**Asp**-Gly-Arg) and basic FGF-(46–53) (Lys-Ser-**Asp**-**Pro**-**His**-**Ile**-Lys-**Leu**), where only conservative amino acid replacements exist in locations of nonidentity. The significance of this observation is unknown.

Computer-assisted protein sequence homology searches (R. F. Doolittle, University of California, San Diego; Protein Sequence Data Base, version 3.0, National Biomedical Research Foundation) have not identified any sequences homologous to that of basic FGF-(1–146). However, amino-terminal microsequence analysis of 750 pmol of [^{14}C]carboxyamidomethylated acidic FGF from bovine brain reveals a striking sequence homology between the basic and acidic FGFs:

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                5        10        15        20        25        30        35
Basic P A L P E D G G S G A F P P G H F K D P K R L Y C K N G G F F L R I H P D G
Acidic                    F N L P L G N Y K K P K L L Y C S N G G Y F L R I L P D G
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Furthermore, we have found that an antiserum raised against a synthetic peptide from basic FGF, [Tyr^{69}]FGF-(69–87) NH_2, crossreacts with acidic FGF (data not shown), strongly suggesting that additional sequence homologies exist between the midportions of these two proteins. The full extent of homology must, of course, await elucidation of the complete structure of acidic FGF, but it is clear that the two major endothelial-cell mitogens are structurally related.

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1. Sullivan, R. & Klagsbrun, M. (1985) *J. Biol. Chem.* **260,** 2399–2403.
2. Maciag, T., Cerundolo, J., Ilsley, S., Kelley, P. R. & Forand, R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5674–5678.
3. D'Amore, P. A. & Klagsbrun, M. (1984) *J. Cell Biol.* **99,** 1545–1549.
4. Maciag, T., Hoover, G. A. & Weinstein, R. (1982) *J. Biol. Chem.* **257,** 5333–5336.
5. Klagsbrun, M. & Shing, Y. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 805–809.
6. Lobb, R. R. & Fett, J. W. (1984) *Biochemistry* **23,** 6295–6299.
7. Bohlen, P., Baird, A., Esch, F., Ling, N. & Gospodarowicz, D. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5364–5368.
8. Thomas, K. A., Rios-Candelore, M. & Fitzpatrick, S. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 357–361.
9. Gospodarowicz, D., Cheng, J., Lui, G., Baird, A. & Bohlen, P. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6963–6967.
10. D'Amore, P. A., Glaser, B. M., Brunson, S. K. & Fenselau, A. H. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3068–3072.
11. Baird, A., Mormede, P. & Bohlen, P. (1985) *Biochem. Biophys. Res. Commun.* **126,** 358–364.
12. Gospodarowicz, D., Cheng, J., Lui, G., Baird, A., Esch, F. & Bohlen, P. (1985) *Endocrinology*, in press.
13. Gospodarowicz, D., Cheng, J., Lui, G. M., Baird, A., Esch, F. & Bohlen, P. (1985) *Endocrinology*, in press.
14. Shing, Y., Folkman, J., Sullivan, R., Butterfield, C., Murray, J. & Klagsbrun, M. (1984) *Science* **223,** 1296–1299.
15. Bohlen, P., Esch, F., Baird, A., Jones, K. L. & Gospodarowicz, D. (1985) *FEBS Lett.* **185,** 177–181.
16. Gospodarowicz, D., Massoglia, S., Cheng, J., Lui, G. M. & Bohlen, P. (1985) *J. Cell. Physiol.* **122,** 323–333.
17. Bohlen, P., Esch, F., Baird, A. & Gospodarowicz, D. (1985) *EMBO J.*, in press.
18. Gospodarowicz, D., Bialecki, H. & Thakral, T. (1979) *Exp. Eye Res.* **28,** 501–514.
19. Bohlen, P., Baird, A., Esch, F. & Gospodarowicz, D. (1985) *J. Cell. Biochem.* **29,** Suppl. 9A, 133 (abstr.).
20. Baird, A., Bohlen, P., Ling, N. & Guillemin, R. (1985) *Regul. Peptides* **10,** 309–317.
21. Gospodarowicz, D., Cheng, J. & Lirette, M. (1983) *J. Cell Biol.* **97,** 1677–1685.
22. Gospodarowicz, D., Massoglia, S., Cheng, J. & Fujii, D. K. (1985) *J. Cell Biol.*, in press.
23. Houghten, R. & Li, C. H. (1983) *Methods Enzymol.* **91,** 549–559.
24. Esch, F. S., Ling, N. C. & Bohlen, P. (1983) *Methods Enzymol.* **103,** 72–89.
25. Bohlen, P. & Schroder, R. (1982) *Anal. Biochem.* **126,** 144–152.
26. Esch, F. (1984) *Anal. Biochem.* **136,** 39–47.
27. Matsuo, H. & Narita, K. (1979) in *Protein Sequence Determination*, ed. Needleman, S. B. (Springer, New York), pp. 104–113.
28. Gospodarowicz, D. (1985) *Methods Enzymol.*, in press.
29. Pierschbacher, M. D. & Ruoslahti, E. (1984) *Nature (London)* **309,** 30–33.
30. Pierschbacher, M. D. & Ruoslahti, E. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5985–5988.
31. Hopp, T. P. & Woods, K. R. (1983) *Mol. Immunol.* **20,** 483–489.
32. Schwarzbauer, J. E., Tamkun, J. W., Lemischka, I. R. & Hynes, R. O. (1983) *Cell* **35,** 421–431.