Hypotensive Activity of Fibroblast Growth Factor

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Acidic and basic fibroblast growth factors (FGFs) are members of a family of proteins that are broad-spectrum mitogens, have diverse hormone-like activities, and function in tumorigenesis. FGF's ability to raise the concentration of intracellular calcium ion suggests that FGF could induce the synthesis of endothelium-derived relaxing factor (EDRF) and consequently vasodilation. Systemic administration of FGF decreased arterial blood pressure. This effect was mediated by EDRF and by adenosine triphosphate–sensitive potassium ion channels. The hypotensive effect of FGF was segregated from its mitogenic activity by protein engineering. These results extend the range of FGF autocrine activities and potential therapeutic applications, emphasize the role of endothelium as an arterial blood pressure–regulating organ, and provide insight on the structural basis of FGF functions.

THE PROTEINS OF THE FGF FAMILY are broad spectrum mitogens synthesized in vertebrates in tissues derived from mesoderm and neuroectoderm (*1*). However, basic FGF (bFGF) also has nonmitogenic activities (*2, 3*). FGF is released by endothelial cells and modulates several mitogenic and nonmitogenic functions of these cells (*2, 4*). Endothelium may influence arterial blood pressure by releasing substances, such as endothelin and EDRF, that control vascular smooth muscle cell tone (*5*). EDRF is synthesized from L-arginine by a Ca^{2+}-activated enzyme system in endothelial cells (*6*), and FGF may increase intracellular Ca^{2+} amounts (*7*). We therefore tested whether systemic administration of FGF to rats and rabbits could decrease arterial blood pressure.

Systemic administration of either acidic FGF (aFGF) or bFGF to anesthetized rats decreased arterial blood pressure in a dose-dependent manner; a maximal response occurred at about 1000 ng per animal (Fig. 1) (*8*). Similar results were obtained both in the presence or absence of heparin at nonsaturating doses of FGF [500 to 900 ng per animal (*9*)]. When bFGF (715 ng) was injected over a period of 1 min, the maximum decrease in pressure was 72% of that observed when the FGF injection was completed in 2 s (as in Fig. 1D). This decrease in the effect of FGF when FGF is administered more slowly is consistent with the reduced amount of FGF expected to accumulate in the blood during the course of a 1-min injection [578 versus 715 ng (*10*)]. Thus, the decrease in blood pressure induced by FGF is probably not caused by transient high local concentrations of the protein (*8*).

Only a single polypeptide was detected in the FGF preparations analyzed by SDS–polyacrylamide gel electrophoresis, reversed-phase high-performance liquid chromatography (HPLC), amino acid analysis (*8*), and NH_2-terminal sequencing. The hypotensive properties of the preparation were destroyed by digestion with chymotrypsin. Thus, the decrease in blood pressure cannot be attributed to nonproteinaceous substances that might be present in the preparation (endotoxins). After repurification by HPLC, aFGF retains its full hypotensive activity (*9*). A hybrid protein that contained the COOH-terminus (118 amino acids) of the enzyme *N*-acetylmuramoyl-L-alanine amidase (autolysin) of *Streptococcus pneumoniae* fused to the NH_2-terminus of aFGF was prepared by protein engineering. The hybrid protein was purified by affinity chromatography on DEAE-cellulose in a single step (*11, 12*) and induced a decrease in the blood pressure equivalent to that caused by aFGF. When the autolysin moiety, produced in the same vector and *Escherichia coli* strain as the hybrid protein and purified by the same procedure, was injected in rats (5 to 40 μg per animal; $n = 4$), the blood pressure remained unaltered (*9*). Thus, the hypotensive effect can be attributed specifically to FGF.

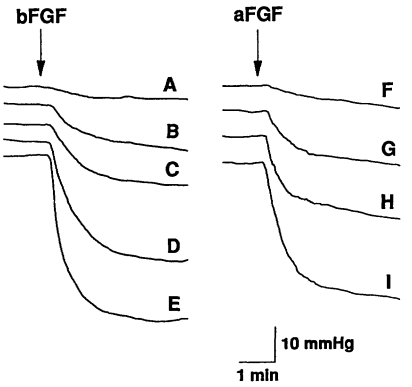

Fig. 1. Effect of intravenous injections of FGF on the mean arterial blood pressure of anesthetized rats. (**A**) 190 ng, (**B**) 323 ng, (**C**) 550 ng, (**D**) 715 ng, and (**E**) 929 ng of bFGF; (**F**) 205 ng, (**G**) 347 ng, (**H**) 587 ng, and (**I**) 985 ng of aFGF. Nearly identical results were found in 90 animals. Arrows indicate time of injection.

A longer term effect of bFGF on blood pressure was studied in rabbits maintained in a steady state of anesthesia (*8*). A half-saturating intravenous dose of bFGF (*8*) reduced blood pressure for at least 20 min after a partial recovery from the initial drop (Fig. 2A). Additional injections of bFGF (3 μg) 20 and 40 min after the initial one caused no further change in blood pressure. However, the hypotensive response to a single injection did not decrease if the experiment was repeated in the same animal 24 hours later. It appears that FGF induced a transient desensitization of the blood pressure–regulating system mediating FGF's hypotensive effect. The initial drop in arterial blood pressure induced by FGF appears to be biphasic (Figs. 1 and 2A). The initial change results from the combination of two processes having apparent first-order kinetics (Fig. 2B). The fast phase represented 60 to 65% of the total change in blood pressure induced by FGF. The half-times ($t_{1/2}$'s) for the fast and slow phases were 2 to 3 and 16 to 19 s, respectively.

To examine whether EDRF participates in FGF-induced hypotension, we investigated the effects of an agent that blocks EDRF synthesis (*13*). Treatment of rabbits with N^{ω}-nitro-L-arginine (L-NNA), an inhibitor of the synthesis of EDRF (*14*), suppressed the slow phase of the initial decrease in blood pressure induced by FGF without affecting the fast phase (during which blood pressure decreased 17 mmHg, equivalent to 65% of the total decrease in the control experiment, with a $t_{1/2}$ of 2.1 s) (Fig. 2C). EDRF may mediate the slow phase of the initial decrease in blood pressure induced by bFGF.

Vasodilation induced by the calcitonin gene–related peptide results from hyperpolarization of cell membranes mediated by adenosine triphosphate (ATP)–sensitive K^+ channels (*15*). Those channels are inhibited by glibenclamide (GB) (*16*). Treatment of the rabbits with GB suppressed the fast phase of the FGF-induced decrease in blood pressure. The slow change was not affected ($t_{1/2}$ of the slow phase was 40 s, representing about 45% of the total pressure decrease of the control) (Fig. 2C). Thus, ATP-sensitive K^+ channels appear to be involved in the fast phase of the initial decrease in blood pressure induced by FGF. The increase of

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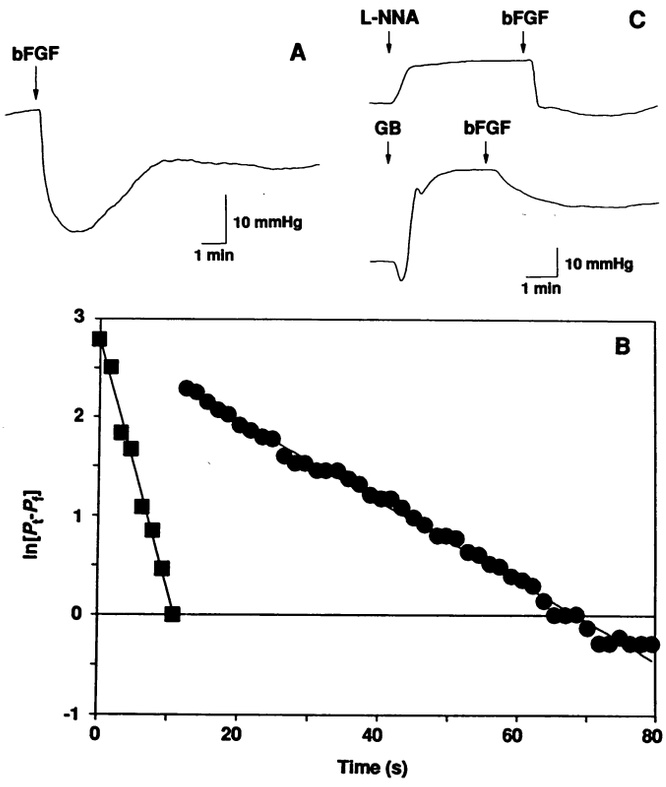

Fig. 2. Long-term effects and involvement of EDRF and ATP-sensitive K^+ channels on the decrease of blood pressure induced by bFGF in anesthetized rabbits. (**A**) Effect of an intravenous injection of bFGF (3 μg at arrow). Similar results were obtained when FGF was injected into the marginal vein of the ear. (**B**) Plot of the two phases of the initial pressure decrease of (A). Time was measured starting at the onset of the pressure deflection. P_t, pressure at time t; P_f, pressure at the end of either the fast (■) or the slow (●) phase. P_f for the fast phase, calculated by iterative optimization, corresponds to 62% of the complete initial drop in pressure. (**C**) Effect of L-NNA and GB on the bFGF-induced (3 μg) decrease in mean arterial blood pressure. Animals were injected at the times indicated with 80 mg of L-NNA in 8 ml of PBS or 12 mg of GB in 4 ml of 25% DMSO in PBS through the contralateral femoral vein and then injected with bFGF at the times indicated. Nearly identical results were found in 17 animals.

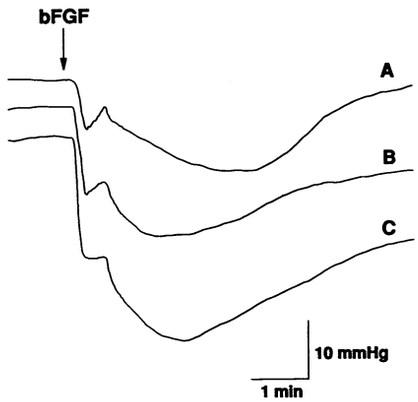

Fig. 4. Effect of bFGF on blood pressure in conscious rabbits. Rabbits (New Zealand White, 2 to 3.5 kg) were injected at the time indicated by the arrow with (**A**) 7 μg, (**B**) 10 μg, and (**C**) 14.4 μg of bFGF in PBS-heparin through the contralateral marginal vein of the ear. Pressure measurements were made through a cannula implanted into the central artery of the ear. The ear used for pressure measurement and FGF injection was anesthetized by injection of its root with 0.25 ml of 2% (v/v) lidocaine.

the $t_{1/2}$ of the slow phase was caused by the solvent used to dissolve GB [dimethyl sulfoxide (DMSO) (25%) in phosphate-buffered saline (PBS)]. The effects of GB andL-NNA imply that hypotensive effects of FGF involve vasodilation. Angiographies (*17*) confirmed that the diameter of the main arteries and their branches increased after injection of FGF (Fig. 3) (*18*).

A truncated form of aFGF, lacking the nuclear translocation sequence at the NH_2-terminus, is mitogenically inactive but still causes early intracellular signaling events (*19*). The difference between the maximum hypotensive effects induced by the injection of equal amounts of this modified protein and the native aFGF were not appreciable in the dose-dependent range [mean difference = 0.675 ± 3.01 mmHg; $n = 4$ (*20*)]. L-NNA and GB had similar inhibitory effects on the actions of both proteins. Therefore, only some of the intracellular events induced by FGF appear to be required for its hypotensive effect.

FGF also had hypotensive effect in conscious rabbits (Fig. 4). However, higher doses of FGF were needed in conscious rabbits to induce equivalent changes in blood pressure than those doses needed in anesthetized rabbits (Fig. 2). Also, a short, transient increase in pressure was observed between the fast and the slow phases of the initial decrease in blood pressure in each experiment with the conscious rabbits. These two differences may be caused by stress responses evoked in the alert animals. During FGF treatments, no alterations in the heart rate or body temperature, signs of tachypnea, or other side effects were observed. Nor did FGF affect adenosine diphosphate– or ristocetin-induced platelet aggregation (*9*).

Our results suggest that FGF may function in regulating blood pressure and that aFGF may control synthesis or release of EDRF in the endothelium by an autocrine mechanism (*4*). However, a more indirect pathway that involves the release of some molecule that acts systemically cannot be excluded. Further research is also necessary to clarify how FGFs activate ATP-sensitive K^+ channels. FGF or FGF-like proteins are often present in tumors and may therefore influence local vasodilation and result in the presence of dilated and leaky vessels that frequently occur in such tissues. Our data may also provide clues to understand hypertension.

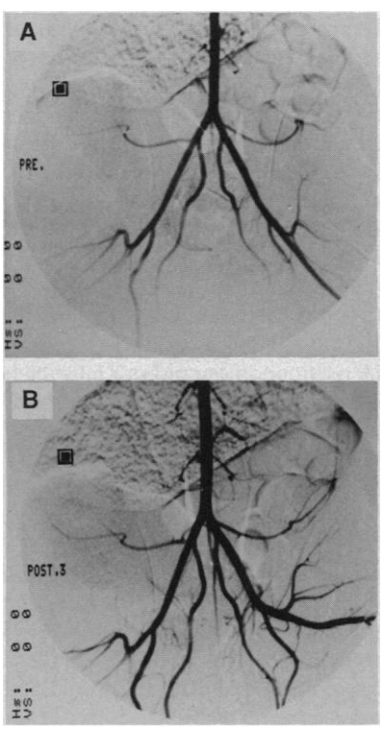

Fig. 3. Effect of bFGF on the diameter of abdominal aorta and iliac arteries and their branches in anesthetized rabbits. (**A**) Before bFGF injection; (**B**) 5 min after injection of bFGF (30 μg).

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8. Human recombinant bFGF (provided by Farmitalia-Carlo Erba, Milan, Italy), did not elicit any appreciable reaction in toxicology studies. The aFGF was prepared as described [D. L. Linemeyer *et al.*, *Biotechnology* **5**, 960 (1987)], except we did not perform HPLC chromatography. Amino acid compositions of aFGF determined by amino acid analysis and deduced from the aFGF sequence, respectively, were as follows: Asp and Asn, 14.52, 15; Thr, 8.02, 8; Ser, 9.87, 10; Glu and Gln, 13.81, 15; Gly, 12.94, 13; Ala, 4.53, 4; Val, 5.20, 5; Met, 2.22, 2; Ile, 5.01, 5; Leu, 16.52, 17; Tyr, 7.88, 8; Lys, 11.00, 11; His, 5.05, 5; Arg, 6.32, 6; Pro, not determined, 8; Cys, not determined, 3; and Trp, not determined, 1. Wistar rats (160 to 200 g) of both sexes were anesthetized by intraperitoneal injection of 3 ml of a mixture per kilogram of body mass; the mixture consisted of ketamine hydrochloride (25 mg/ml), atropine (0.1 mg/ml), and Valium (2 mg/ml). For arterial blood pressure measurements, the common femoral artery was cannulated, and the catheter was moved into the abdominal aorta. The pressure was recorded on a monitor connected to the catheter by a pressure transducer. Pressure values at intervals of 1.6 s were averaged manually and digitized for computer analysis and graphing. FGF was injected into the external jugular vein in a single bolus (50 μl) in PBS with heparin (0.1 mg/ml) (PBS-heparin), except where indicated. Similar results to those of Fig. 1 were obtained when the injection was carried out in the contralateral common femoral artery or when the injection volume was brought up to 500 μl. New Zealand white rabbits (2 to 3.5 kg) of both sexes were sedated with 10 mg of ketamine hydrochloride (per kilogram of body mass) given through the ear marginal vein. Anesthesia was maintained with 1 to 3% halothane and a mixture of N_2O:O_2 (3:1). The FGF was injected in the contralateral femoral vein in a single 500 μl bolus in PBS-heparin.
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$$F = -(K_2/K_1)(1 - e^{K_1 t}) + K_3$$

F, the total amount of FGF in the blood at time t; K_2, the FGF injection rate; K_1, the FGF distribution constant; and K_3, the FGF amount that remains in the blood after the distribution phase, which in the time range of our calculations can be assumed as constant.
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17. We sedated New Zealand white rabbits (2 to 3.5 kg) with ketamine hydrochloride and implanted a catheter near the bifurcation of the abdominal aorta through the left common carotid artery for the injection of FGF and contrast media to make the arteries opaque. Automatic injection of contrast media (3 ml total at 1 ml/s) was electronically synchronized with the digital vascular imaging equipment. The angiographies were obtained 5 s after the onset of injection. FGF was injected in 500 μl of PBS-heparin.
18. Mean cross-sectional increase of large and medium size arteries after FGF injection (Fig. 3) was estimated at 41%.
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20. The decrease in blood pressure induced in anesthetized rabbits (*8*) by different amounts (micrograms of protein per animal) of native aFGF and the modified protein, respectively, were as follows: 1.4 μg of protein, 8.6 and 6.5 mmHg; 5.4 μg of protein, 17.8 and 15.4 mmHg; 10.7 μg of protein, 21.1 and 25.7 mmHg; and 21.4 μg of protein, 23.7 and 26.3 mmHg.
21. All animals were kept under the care of the vivarium staff and treated according to protocols approved by our institutional animal research committee. We thank R. Guillemin, J. M. Ramírez, and M. Nieto-Sampedro for comments; and B. Cuevas and A. Crespo for technical assistance. Partially supported by the Dirección General de Investigación Científica y Técnica.

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DNA Bending by Fos and Jun: The Flexible Hinge Model

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DNA bending is essential for the assembly of multiprotein complexes that contact several DNA sequence elements. An approach based on phasing analysis was developed that allows determination of both the directed DNA bend angle and the orientation of DNA bending. This technique has been applied to the analysis of DNA bending by the transcription regulatory proteins Fos and Jun. Complexes that contained different combinations of full-length and truncated Fos and Jun induced DNA bends of different magnitudes and orientations. The DNA bends induced by the individual proteins were determined on the basis of a quantitative model for DNA bending by dimeric complexes. This information was used to visualize the consequences of DNA bending by Fos and Jun for the structures of Fos-Jun-DNA and Jun-DNA complexes.

EUKARYOTIC GENE TRANSCRIPTION IS modulated by combinatorial interactions among sequence-specific DNA binding proteins (*1*). For interactions to occur between proteins bound to separate sequence elements, the DNA helix must often be distorted. Protein-induced DNA bending can participate in the regulation of transcription by facilitating assembly of initiation complexes (*2*). Thus, it is important to determine the orientation and magnitude of DNA bends induced by transcriptional regulatory proteins.

The proto-oncogenes c-*fos* and c-*jun* encode proteins that are members of the bZIP family of DNA binding proteins, which bind DNA as homo- or heterodimeric complexes (*3*). Dimerization is mediated by a leucine zipper interaction, and DNA binding requires an adjacent region that contains a high density of basic amino acids (*4*). This basic DNA binding domain adopts an α-helical structure upon binding to DNA (*5*). Models of the DNA binding complexes of bZIP proteins assume that the DNA binding domain interacts with a straight B-form DNA (B-DNA) recognition site (*6, 7*). However, contacts between a straight α helix and the major groove of straight B-DNA are limited to a maximum of 12 contiguous amino acids, which can contact a maximum of 5 bp on DNA. In contrast, the basic region extends over 20 residues, and the DNA contact regions for proteins in the bZIP family range between 12 and 16 bp (*8*). Thus, the basic region α helix or the DNA recognition site or both must be bent or distorted to allow for the observed regions of contact between the molecules.

Procedures have been developed to investigate protein-induced DNA bending that rely on the anomalous electrophoretic mobilities of bent DNA fragments (*9, 10*). Using these methods, we demonstrated that Fos-Jun heterodimers and Jun homodimers induce bends in opposite orientations and that complexes composed of peptides encompassing the dimerization and DNA binding domains bend DNA in the same

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