Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 1513–1517, February 1993
Medical Sciences

Perivascular and intravenous administration of basic fibroblast growth factor: Vascular and solid organ deposition

ELAZER R. EDELMAN*†‡, MATTHEW A. NUGENT†, AND MORRIS J. KARNOVSKY§

*Cardiovascular Division, Department of Internal Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115; †Harvard University–Massachusetts Institute of Technology, Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139; and §Department of Pathology, Harvard Medical School, Boston, MA 02115

Communicated by Robert Langer, November 18, 1992 (received for review August 31, 1992)

ABSTRACT **The *in vivo* mitogenicity of basic fibroblast growth factor (bFGF) for arterial smooth muscle cells relies on the removal of endothelium, raising the question of whether the endothelium serves as a mechanical barrier preventing contact of circulating bFGF with underlying smooth muscle cells or as a biochemical barrier that produces a local inhibitor of bFGF activity. To better define the role of the intact endothelium in modulating the vascular and tissue deposition of bFGF, we compared the fate of intravenous injections of ^{125}I-labeled bFGF with perivascular controlled growth factor release. Peak serum bFGF levels were detected within 1 min of injection, and the growth factor was cleared thereafter with a serum half-life of almost 3 min. Polymeric controlled release devices delivered bFGF to the extravascular space without transendothelial transport. Deposition within the blood vessel wall was rapidly distributed circumferentially and was substantially greater than that observed following intravenous injection. The amount of bFGF deposited in arteries adjacent to the release devices was 40 times that deposited in similar arteries in animals who received a single intravenous bolus of bFGF. Endothelial denudation had a minimal effect on deposition following perivascular release, and it increased deposition following intravenous delivery 2-fold. The presence of intimal hyperplasia increased deposition of perivascularly released bFGF 2.4-fold but decreased the deposition of intravenously injected bFGF by 67%. In contrast, bFGF was 5- to 30-fold more abundant in solid organs after intravenous injection than it was following perivascular release. Deposition was greatest in the kidney, liver, and spleen and was substantially lower in the heart and lung. Thus, bFGF is rapidly cleared following intravenous injection and is deposited within both solid organs and the walls of blood vessels. Unlike the mitogenic potential of bFGF within blood vessels, vascular deposition is virtually independent of the presence of endothelium. Perivascular delivery is far more efficient than intravenous delivery at depositing bFGF within the arterial wall, and an increased neointima may provide added substrate for potential bFGF deposition but has limited contact with intravascular growth factor as a result of dilutional and flow-mediated effects.**

Basic fibroblast growth factor (bFGF) is synthesized by and mitogenic for a variety of cell types, including endothelial cells and smooth muscle cells, and is intensely angiogenic (1–5). Intravenous infusion of bFGF stimulates endothelial regeneration (6) and smooth muscle cell proliferation (7) after endothelial denudation. The effect on smooth muscle cells was noted only in the absence of endothelium, and it was concluded that the endothelium may serve as a barrier preventing contact of the mitogen, bFGF, with the target, vascular smooth muscle cells (7). We confirmed that bFGF was both angiogenic and mitogenic for vascular smooth muscle cells *in vivo* and demonstrated that these two effects were coupled (8). The extravascular release of bFGF was especially potent at stimulating smooth muscle cell proliferation, but once again only in the absence of endothelial cells (8). Thus, at a time when contact of bFGF with vascular smooth muscle cells might occur without traversing the endothelium, persistent inhibition of bFGF activation in the presence of an intact endothelium may imply that the endothelium is serving as more than a physical barrier. The antiproliferative and growth factor-binding aspects of endothelium-derived heparan sulfate proteoglycan make this and related compounds prime candidates for biochemical regulators of this aspect of bFGF vascular biology (9–11).

To better define the role of the intact endothelium in modulating the vascular and tissue deposition of bFGF, we compared the fate of intravenous injections of radiolabeled bFGF with perivascular controlled growth factor release. Longitudinal and circumferential depositions of bFGF in rats were followed and compared for intravenous injection and perivascular delivery in native arteries with an intact architecture, arteries subjected to endothelial denudation, and arteries where endothelial denudation was followed by substantial neointimal hyperplasia and smooth muscle cell proliferation. bFGF was rapidly cleared from serum after intravenous injections, and deposited to a greater extent in solid organs than in blood vessel walls. Vascular deposition increased only 2.2-fold upon removal of the endothelium and decreased 67% in the presence of intimal smooth muscle cell proliferation. In comparison to intravenous injections, continuous perivascular release was accompanied by decreased circulating levels, lower deposition within solid organs, and substantially increased deposition within arterial walls.

METHODS

bFGF. Purified recombinant human bFGF corresponding to the 146-amino acid form of the growth factor (12, 13) was from Chiron. ^{125}I-bFGF was prepared (12), with specific radioactivity ranging from 25 to 100 nCi/ng (1 nCi = 37 Bq). Trichloroacetic acid precipitation (12.5%, wt/vol; 1 hr at 4°C) and SDS/PAGE validated the stability and integrity of the radiolabeled growth factor. Samples of stock solutions of ^{125}I-bFGF stored at −20°C were compared with ^{125}I-bFGF released from alginate microspheres at 37°C under shaking conditions for 2 weeks. There was no difference in the amount of radioactivity precipitated from the stocks and release media. In addition, 66.4 ± 0.1% of the radioactivity in the stock solution of ^{125}I-bFGF and 63.4% of the radioactivity in the release media localized to a single band in SDS/polyacrylamide gels (4–14% gradient) at 18 kDa.

Intravenous Injection of bFGF. bFGF was mixed with trace amounts of ^{125}I-bFGF to produce a final bFGF concentration of 1.67 μg/ml in 0.9% NaCl/1 mM $CaCl_2$/0.05% gelatin, with

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: bFGF, basic fibroblast growth factor.

‡To whom reprint requests should be addressed at: Department of Pathology, D2-336, Harvard Medical School, Boston, MA 02115.

a specific activity of 1.3–1.5 × 10^7 cpm/μg. Approximately 0.2–0.3 μg of bFGF was injected into the jugular vein of rats. The precise amount delivered to the circulation of each animal was determined by subtracting the amount of radioactivity retained in the vessel containing the bFGF solution and in the syringe used from the initial total amount present in the container.

Perivascular bFGF Release. bFGF was ionically bound to heparin-Sepharose beads (Pharmacia LKB) and encapsulated within calcium alginate microcapsules (8, 14). This provided a stable platform for the bFGF, enabled prolonged storage, and established a means for the controlled release of bFGF to specific sites *in vivo*. Heparin-Sepharose beads (3.33 mg/ml) were sterilized under ultraviolet light for 30 min and then mixed with filter-sterilized sodium alginate (1.2%, wt/vol; Sigma). The mixed slurry was dropped through a needle into a beaker containing a hardening solution of $CaCl_2$ (1.5%, wt/vol). Microcapsules were formed instantaneously. Uniformly crosslinked capsule envelopes were obtained by incubating the capsules in the $CaCl_2$ solution first for 5 min under gentle mixing, and then for 10 min without mixing. The microcapsules were then washed three times with sterile water and stored in 0.9% NaCl/1 mM $CaCl_2$ at 4°C for an indefinite period of time without degradation (8, 14). Each microcapsule in its hydrated state contained 0.05 mg of heparin-Sepharose, 0.18 mg of alginate, and 11 mg of water. bFGF was incorporated within the microcapsules, after calcium alginate matrix formation and hardening, by incubation in 0.9% $CaCl_2$/1 mM $CaCl_2$/0.05% gelatin with bFGF and trace amounts of ^{125}I-bFGF (10 μl per microcapsule) for 16 hr under gentle agitation at 4°C. *In vitro* release kinetics were defined by examining microcapsules residing within a large volume (2 ml per bead) of 150 mM NaCl/1 mM $CaCl_2$/0.05% gelatin, pH 7.4, under gentle shaking at 37°C. Mass balance determinations were used to establish *in vivo* release rates.

Arterial Injury. Endothelial denudation of the left common carotid artery in male Sprague–Dawley rats (300–500 g; Charles River Breeding Laboratories) was performed with a 2 French Fogarty balloon catheter (American Edwards Laboratories, Santa Ana, CA), under intraperitoneal sodium pentobarbital (Nembutal, 0.05 mg/g of body weight) anesthesia (8, 15, 16). The contralateral artery underwent identical manipulation save for the introduction of the balloon catheter. Microcapsules were placed adjacent to the arteries and maintained in place by closing fascial planes over the artery (8, 16). bFGF was released from these devices directly to the arterial wall. Tissue harvest was performed 1 hr after intravenous injection and 2.3 hr after microsphere placement, as these times were demonstrated in preliminary experiments to provide similar circulating levels of bFGF for the two modes of growth factor administration. At earlier times serum bFGF levels following intravenous injection were higher than that obtained with perivascular release, and at later times lower than those observed with perivascular release. Animals were euthanized and perfused clear retrogradely via the abdominal aorta with lactate-containing Ringer's solution followed by modified Ito–Karnovsky's or Carnoy's fixative. The location of the implanted devices was noted and the devices were recovered with the intact arteries. The extent of arterial injury was defined histologically. Fixed sections were paraffin-embedded, microtome-sectioned, and stained with verHoeff's elastin stain. Intimal and medial areas and their ratio was determined with the aid of a semiautomated computer-guided microscopic planimeter.

Plasma Concentrations and Tissue Deposition. bFGF deposition was defined in six experimental groups of eight arteries each, including animals treated with intravenous or perivascular bFGF, and arteries subjected to (*i*) no injury, (*ii*) endothelial denudation, or (*iii*) intimal hyperplasia 2 weeks after deendothelialization. Arterial and venous blood were obtained from the descending aorta and the inferior vena cava just below the renal arteries in all animals immediately before pressure perfusion. After perfusion, tissue samples were excised from the liver, spleen, kidney, myocardial apex, lung, aorta, femoral artery, and both carotid arteries. In four separate animals circumferential distribution was determined from arterial sections and adjacent embedded microspheres excised *en bloc*. The arteries were mounted and pinned on dental wax in their *in vivo* orientation and then cut into 3-mm rings. The center 3-mm rings in contact with the microspheres were further divided in quadrants.

Fixed tissue samples were air-dried, weighed, and measured, and ^{125}I-bFGF was determined in a gamma counter (LKB). Background was subtracted, and the amount of bFGF deposited within a given sample was calculated from the specific activity of the labeled bFGF. Results were expressed as picograms deposited per microgram injected per milligram of tissue retrieved. This calculation normalized data for changes in the amount of bFGF injected or released and for differences in tissue sample weight.

In three additional animals, polyethylene tubing (PE-50; Clay-Adams–Becton Dickinson) was inserted via the external carotid arteriotomy and sutured in place to allow for continuous monitoring of arterial blood. The catheter tip did not extend beyond the carotid bifurcation. Catheters drained into glass test tubes rotating underneath at a fixed rate. The tubes rested at a fixed height below the animal such that flow rates were matched for all animals tested. This was confirmed by identity of the volumes collected.

RESULTS

Plasma Levels. When a catheter was inserted within the external carotid artery and blood was sampled continuously after injection, the serum kinetics displayed in Fig. 1 were obtained. Approximately 60% of the injected bFGF, 3.8 ng/ml, was detected in the serum 1 min after intravenous injection. First-order clearance was noted thereafter with a serum half-life of 174.3 sec (2.9 min). Tissue was harvested 1 hr after injection, and arterial and venous concentrations of 2.3 ± 0.3 and 2.4 ± 0.3 pg/ml were noted.

Alginate microspheres contained bFGF at 423.9 ± 19.6 ng. Release profiles followed those previously reported for these systems (14), and within the 2.3-hr course of the *in vivo* experiments 14.4 ± 0.6% of the bFGF was released, at 26.5 ± 3.4 ng/hr. The *in vivo* release rate was 73.7 ± 6.0% of that observed for identical microspheres releasing under mixing conditions at 37°C *in vitro*. At this *in vivo* release rate, circulating bFGF levels at tissue harvest were similar to those

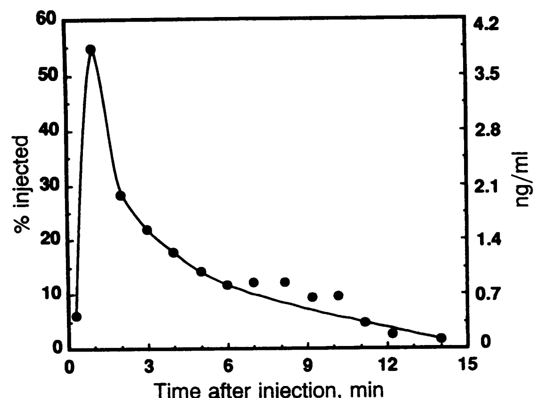

FIG. 1. Continuous determination of plasma arterial bFGF was performed on three animals, and the average data are displayed as percentage of injected bFGF and as absolute concentration (ng/ml). Levels rose within 1 min of injection, and first-order clearance with a serum half-life of 174.3 sec was observed thereafter.

detected for intravenous release. Little to no bFGF was detected within the first 15 min of microsphere release, but at tissue harvest, 2.3 hr after implantation, arterial and venous levels of 3.9 ± 0.2 and 3.8 ± 0.3 pg/ml were obtained.

Lateral Deposition. Perivascular release of bFGF was an efficient means of depositing growth factor within the blood vessel wall. Determination of longitudinal and circumferential distribution of bFGF within the blood vessel revealed that 80.0 ± 3.8% of the released bFGF was restricted to the 3-mm-long segment of artery immediately adjacent to the microsphere, and the remaining bFGF was distributed in the 3-mm segments immediately above and below, with twice as much in the latter (Fig. 2). This presumably represents the effects of blood flow on bFGF distribution. When the point of contact of the microsphere with the adjacent arterial segment was marked and the arterial segment was cut in quadrants, the circumferential distribution of growth factor was noted (Fig. 2). Within 2.3 hr of release initiation, bFGF was noted throughout the circumference of the segment. Though 58.8 ± 4.3% of the growth factor was in the portion of the artery in immediate contact with the microsphere, adjacent quadrants had 15–20%, and the quadrant opposite the microsphere had about 5% of the deposited bFGF.

Tissue Deposition. Perivascular delivery of bFGF was substantially more efficient at depositing bFGF within vascular structures than intravenous delivery (Fig. 3). Arteries adjacent to the release devices had 40.0 times more bFGF than similar arteries from animals who received a single intravenous bolus of bFGF ($P < 0.006$). When these arteries were denuded of endothelium there was a statistically insignificant 10% decrease in deposition following perivascular release and a 2.0 ± 0.5-fold increase for intravenous injection (Fig. 3). Though removal of the endothelium increased deposition after intravenous injection ($P < 0.005$), perivascular delivery was still 18.9 times as efficient at depositing bFGF within the artery ($P < 0.0001$). Arteries distant from the perivascular implants also had increased deposition following perivascular release. When the microspheres were placed around the carotid arteries, there was 3.2-fold greater deposition within the aorta ($P < 0.007$) and 2.7-fold greater deposition within the femoral arteries ($P < 0.044$) than that seen in the aorta and femoral arteries of animals who received injections of bFGF (Fig. 4).

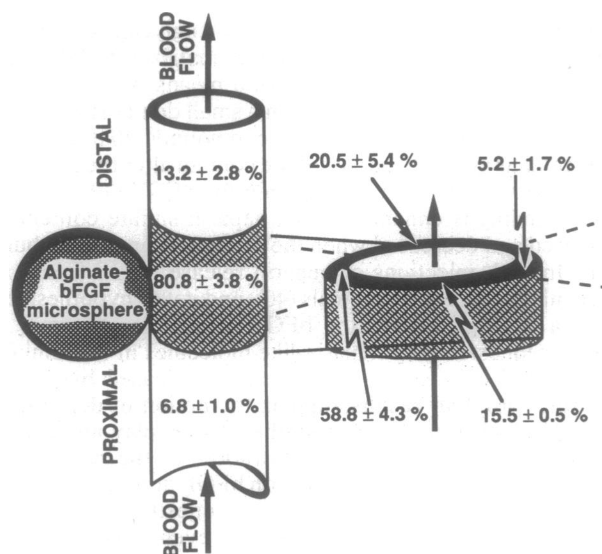

FIG. 2. Lateral and circumferential distribution of bFGF after release from alginate microspheres was determined for four microspheres placed adjacent to common carotid arteries of laboratory rats. The position of the microspheres was marked on the arteries during harvest 2.3 hr after implantation. Deposition was determined for the 3-mm arterial segments immediately in contact with the alginate release device and for 3-mm segments immediately proximal and distal to that. The contact segments were further subdivided into quadrants. Data are expressed as percentage of total deposited bFGF present in an individual arterial section (mean ± SEM, n = 4).

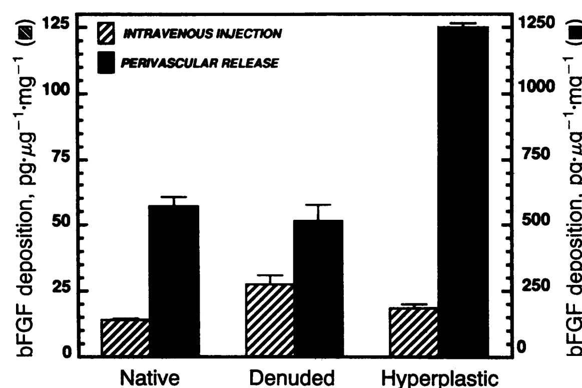

FIG. 3. The deposition of bFGF within the common carotid artery in its native state with intact endothelium (n = 8) is contrasted with the deposition in endothelium-denuded arteries (n = 8) and in arteries allowed to develop intimal hyperplasia 2 weeks after endothelial denudation (n = 8). bFGF was provided by a single intravenous injection (hatched bars) or by perivascular release (solid bars). Deposition of bFGF after perivascular release was not statistically altered immediately after endothelial denudation. However, if bFGF release was initiated 2 weeks after injury, at a time when neointimal hyperplasia had occurred, deposition was increased 2.2-fold ($P < 0.002$ compared with native artery). In contrast, deposition of bFGF after intravenous injection was substantially lower than with perivascular release for native arteries (factor of 40.0 less), denuded arteries (factor of 18.9 less), and hyperplastic arteries (factor of 67.1 less). Endothelial denudation was accompanied by a 1.9-fold increase in deposition above that seen in the native artery ($P < 0.005$), while the presence of intimal hyperplasia actually muted this increase 67%, so that it was only 1.3 times the deposition in native artery ($P < 0.02$).

When bFGF administration was delayed 2 weeks after balloon injury, intimal hyperplasia within the denuded arteries was maximized, and bFGF deposition following intravenous injection was 67% lower than that noted when the growth factor was injected immediately upon denudation ($P < 0.05$; Fig. 3, hatched bars). In contrast, deposition of bFGF from perivascular controlled release devices placed 2 weeks after injury was 2.4-fold greater than that observed when the devices were implanted immediately after injury ($P < 0.0005$; Fig. 3, solid bars). There was no difference between the two groups in the amount of intimal hyperplasia induced by

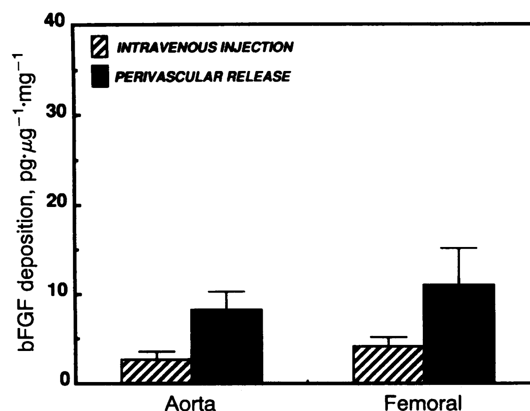

FIG. 4. Deposition of bFGF within the wall of the descending abdominal aorta and the femoral arteries was 3.2- and 2.8-fold greater after release of growth factor into the perivascular space of the left carotid artery when compared with a single intravenous injection.

balloon injury. The intima/media area ratio in the intravenously treated groups was 1.131 ± 0.07, and that in the perivascularly treated group was 1.135 ± 0.08. Thus, perivascular release was 67.9 ± 0.1 times more effective in depositing bFGF in hyperplastic arteries than intravenous injection ($P < 0.0001$) and as a result solely of increase in deposition and not because of a difference in intimal mass. The 2-week hiatus had no effect on the solid organ, femoral artery, or aortic deposition for both types of delivery and in the common carotid artery contralateral to the injured artery after intravenous injection.

The deposition of bFGF within solid organs followed a reverse trend. bFGF was 5- to 30-fold more abundant in solid organs after intravenous injection than it was following perivascular release (Fig. 5). Deposition was greatest in the kidney, liver, and spleen and was substantially lower in the heart and lung.

DISCUSSION

Removal of endothelium is an essential requirement for the mitogenecity of bFGF within the blood vessel wall of the rat (7, 8). In the presence of an intact endothelium neither intravenous injection (7) nor periarterial release (8) of bFGF had a demonstrable biologic effect on blood vessels. When arteries were denuded of endothelium, neointimal hyperplasia (7, 8) and perivascular angiogenesis (8) were increased by bFGF. It had been surmised that the endothelium may prevent contact between circulating growth factor and target smooth muscle cells and that removal of the endothelium removed this mechanical barrier (7). Yet the observation that endothelial removal was still required for bFGF activation when the growth factor was released from the adventitial aspect of the artery indicated that mechanical intervention alone might not account for the full extent of the endothelial effect. The results reported in this paper confirm that bFGF is rapidly cleared from serum after intravenous injection and demonstrate deposition within both solid organs and the arterial wall, but in a manner relatively independent of the vascular endothelium. Deposition of bFGF within different tissues was determined more by mode of administration than by the presence or state of the endothelial cells. Perivascular release of bFGF was more effective at establishing vascular deposition of bFGF, near and far from the release device, and intravenous delivery was more effective at deposition within solid organs. Deposition was inconsequentially increased by endothelial denudation. Thus, the possibility that the endothelium is serving only as a mechanical barrier between blood in the lumen and medial smooth muscle cells seems remote, and the inhibitory function of the endothelium must stem from more complex phenomena.

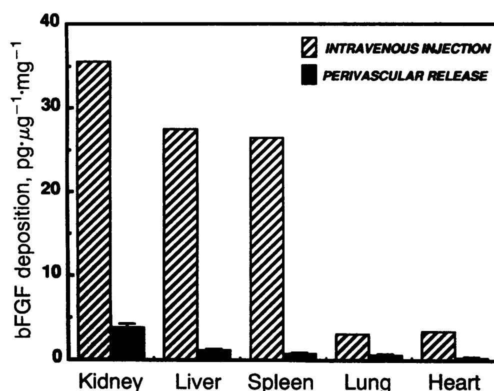

FIG. 5. Intravenous injection of bFGF led to a 4–30 times greater deposition of growth factor within the kidney, liver, spleen, lung, and heart than when the growth factor was provided by controlled release from perivascular microspheres.

The interaction between vascular endothelial cells and smooth muscle cells is multidimensional. Indeed, the tone (17–20) and thromboresistance of the blood vessel, as well as its proliferative state, are regulated by sophisticated interactions between these two cell types. In the native state the intact layer of endothelial cells maintains underlying smooth muscle cells quiescent. Removal of the endothelium exposes underlying matrix and smooth muscle cells and is accompanied by a cascade of events that leads to thrombosis (21) and to vascular smooth muscle cell proliferation. Restoration of the endothelial lining after vascular denudation enhances regression of smooth muscle cell proliferation (22). Confluent endothelial cells in culture produce a heparin-like compound that is inhibitory for smooth muscle cells in tissue culture (23, 24), and exogenous heparin preparations inhibit smooth muscle cell proliferation within the neo-intima of arteries following endothelial injury (25). Proliferating endothelial cells, on the other hand, secrete a factor(s) that stimulates smooth muscle cell growth (26–28), as well as a number of smooth muscle cell mitogens including bFGF and platelet-derived growth factor (29–32). These same growth factors bind avidly to the heparin-like antiproliferative compounds, and the activation of bFGF requires the presence of heparan sulfate proteoglycan (33, 34). Thus, it is possible that a balance between endothelium-derived mitogens and inhibitory factors governs smooth muscle cell homeostasis. In the quiescent state the inhibitory factors may be present in abundance, suppressing smooth muscle proliferation directly or preventing mitogenic activity by sequestering potential mitogens from the vascular smooth muscle cells. Our data suggest that these factors might make use of both of these mechanisms.

The deposition of growth factors in arteries or solid organs might serve not only to sequester bFGF but also as a means of concentrating bFGF within the local pericellular environment and/or as a scaffolding from which the growth factor might ultimately be continuously released. The kinetics of bFGF in the pericellular environment may involve repeated binding and release both from the bFGF tyrosine kinase receptor and from heparan sulfate proteoglycan on the cell surface and within the extracellular matrix (35–38). This mechanism has been postulated as a means of maintaining multiple molecules of bFGF within a small domain of binding sites (38). Soluble heparin and compounds that regulate extracellular matrix heparan sulfate content alter the capacity of each extracellular matrix domain for bFGF (39), so that bFGF binding is enhanced when heparin sulfate content is increased and decreased when the proteoglycan is less abundant. Indeed, injections of heparin release bFGF from extracellular matrix in lab animals (40), and it was hypothesized that "a large portion of the bFGF in the blood stream is rapidly sequestered by heparin-like molecules in the luminal surface of the endothelium and can be released by native heparin" (40). Our results support the notion of deposition but indicate that other, nonendothelial sites play an equally important role in potential sequestration, as the removal of endothelium had little to no affect on bFGF deposition within the arterial wall. It is possible that sequestration is to the basement membrane, elastic lamina, or even smooth muscle cells within the media. These structures contain glycosaminoglycans and proteins (collagen, fibronectin, laminin) that are capable of binding heparin and heparin-avid growth factors (41). Local injury or removal of the endothelium may lead to the removal of endothelium-derived smooth muscle cell growth inhibitors, loss of cell growth control, heightened local release of mitogens, and stimulation of endothelial and smooth muscle cell growth (12, 42, 43). When the mass of endothelial cells is great enough the inhibitory products and functions may once again predominate.

That bFGF deposition within the hyperplastic artery was increased above that observed in native or denuded arteries after perivascular release and decreased following intravenous injection may indicate a complex relationship between the amount of potential bFGF binding tissue and flow-mediated effects. The segment of the artery proximal to the bFGF-releasing microsphere had half as much bFGF as was deposited within the distal segment (Fig. 2). Thus, one model might envision that intimal hyperplasia and the accompanying increase in vascular smooth muscle cell mass and extracellular matrix provide an increased substrate for bFGF binding. This substrate, however, requires adequate contact between the growth factor and its binding sites. If increased and turbulent flow carries the compound away, as may be the case for circulating bFGF, relatively less bFGF may come in contact with the artery and less bFGF may be deposited per mass of arterial tissue. In contrast, perivascular release has none of the constraints of flow dilution. bFGF pools around an artery after release from the controlled delivery device; capillary absorption may absorb the growth factor, but the marked dilutional affect of a large volume of rapidly flowing blood is absent. Capillary uptake and other phenomena may in fact expedite transport of the growth factor throughout the hyperplastic segment of the wall and to distant vascular sites.

Our data may provide evidence for specialized transvascular transport that provides for enhanced or expedited conveyance of compounds along the vascular chain from one site to another. Not only was circumferential distribution of the released bFGF rapid (Fig. 2), but at a time when the solid organ deposition of bFGF released from devices alongside the carotid arteries was lower by a factor of 5–30 than that achieved with intravenous injection, deposition of bFGF was 3-fold higher in arterial structures distant from the source of release and the carotids. It has long been hypothesized that the vasa vasorum play a role in transport of compounds to the blood vessel wall from distant sites (44). bFGF increases vasa vasorum density around injured arteries (8, 45). Structures that, like the vasa vasorum, run alongside major arteries and atherosclerotic vessels may provide for increased deposition of bFGF along the vasculature and within the blood vessel wall.

bFGF was rapidly cleared following intravenous injection and deposited within both solid organs and the walls of blood vessels. The serum half-life we obtained is very close to that previously reported (46). Taken together with the extent of deposition of the growth factor within the various organs, these data have profound implications for studies using intravenous administration of bFGF. If serum levels alone dictate the availability of biologically active bFGF, a more sustained form of administration should be required. Unlike the activation of bFGF's vascular mitogenic effects, vascular deposition is virtually independent of the endothelium. Perivascular delivery is far more efficient than intravenous delivery at depositing bFGF within the arterial wall, and an increased neointima may provide added substrate for potential bFGF deposition which may be diluted by intravascular mixing with blood and removed with continuous blood flow.

We thank Amy Newton for technical assistance. This work was supported by a grant from the National Institutes of Health (HL17747), a Physician–Scientist Award of the National Institutes of Health (K12 AG00294) to E.R.E., and a National Institutes of Health fellowship (F32 GM14003) to M.A.N.

1. Montesano, R., Vassali, J. D., Baird, A., Guillemin, R. & Orci, L. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7287–7301.
2. Connolly, D. T., Stoddard, B. L., Harakas, N. K. & Feder, J. (1987) *Biochem. Biophys. Res. Commun.* **144**, 705–712.
3. Hayek, A., Culler, F. L., Beattie, G. M., Lopez, A. D., Cuevas, P. & Baird, A. (1987) *Biochem. Biophys. Res. Commun.* **147**, 876–880.
4. Thompson, J. A., Anderson, K. D., DiPietro, J. M., Zwiebel, J. M., Zametta, M., Anderson, W. F. & Maciag, T. (1988) *Science* **241**, 1349–1352.
5. Presta, M., Maier, J. A. M., Rusnati, M. & Ragnotti, G. (1989) *J. Cell. Physiol.* **140**, 68–74.
6. Lindner, V., Majack, R. A. & Reidy, M. A. (1990) *J. Clin. Invest.* **85**, 2004–2008.
7. Lindner, V., Lappi, D. A., Baird, A., Majack, R. A. & Reidy, M. A. (1991) *Circ. Res.* **68**, 106–113.
8. Edelman, E. R., Nugent, M. A., Smith, L. T. & Karnovsky, M. J. (1992) *J. Clin. Invest.* **89**, 465–471.
9. Wight, T. (1989) *Arteriosclerosis* **9**, 1–20.
10. Ruoslahti, E. & Yamaguchi, Y. (1991) *Cell* **64**, 867–869.
11. Jackson, R. L., Busch, S. J. & Cardin, A. D. (1991) *Physiol. Rev.* **71**, 481–539.
12. Bashkin, P., Doctrow, S., Klagsbrun, M., Svahn, C. M., Folkman, J. & Vlodavsky, I. (1989) *Biochemistry* **28**, 1737–1743.
13. Klagsbrun, M. & Edelman, E. R. (1989) *Arteriosclerosis* **9**, 269–278.
14. Edelman, E. R., Mathiowitz, E., Langer, R. & Klagsbrun, M. (1991) *Biomaterials* **12**, 619–626.
15. Clowes, A. W., Reidy, M. A. & Clowes, M. M. (1983) *Lab. Invest.* **49**, 327–333.
16. Edelman, E. R., Adams, D. A. & Karnovsky, M. J. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3773–3777.
17. Busse, R., Trogisch, G. & Bassenge, E. (1985) *Basic Res. Cardiol.* **80**, 475–490.
18. De Mey, J. G. & Vanhoutte, P. M. (1982) *Circ. Res.* **51**, 439–447.
19. Furchgott, R. F. & Zawadzki, J. V. (1980) *Nature (London)* **288**, 373–376.
20. Furchgott, R. F. & Vanhoutte, P. M. (1989) *FASEB J.* **3**, 2007–2017.
21. Fuster, V., Steele, P. M. & Chesebro, J. H. (1985) *J. Am. Coll. Cardiol.* **5**, 175B–184B.
22. Bjornsson, T. D., Dryjski, M., Tluczek, J., Mennie, R., Ronan, J., Mellin, T. N. & Thomas, K. A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 8651–8655.
23. Castellot, J. J. J., Addonizio, M. L., Rosenberg, R. D. & Karnovsky, M. J. (1981) *J. Cell Biol.* **90**, 372–379.
24. Castellot, J. J. J., Cochran, D. L. & Karnovsky, M. J. (1985) *J. Cell. Physiol.* **124**, 21–28.
25. Clowes, A. W. & Karnovsky, M. J. (1977) *Nature (London)* **265**, 625–626.
26. Williams, S. K. (1991) *Lab. Invest.* **64**, 721–723.
27. Koo, E. W. & Gotlieb, A. I. (1991) *Lab. Invest.* **64**, 743–753.
28. Koo, E. W. & Gotlieb, A. I. (1989) *Am. J. Pathol.* **134**, 497–503.
29. Miossec, P., Cavender, D. & Ziff, M. (1986) *J. Immunol.* **136**, 2486–2491.
30. Greisler, H. (1989) *J. Vasc. Surg.* **10**, 566–568.
31. Vlodavsky, I., Friedman, R., Sullivan, R., Sasse, J. & Klagsbrun, M. (1987) *J. Cell. Physiol.* **131**, 402–428.
32. Schweigerer, L., Nuefeld, G., Friedman, J., Abraham, J. A., Fiddes, J. C. & Gospodarowicz, D. (1987) *Nature (London)* **325**, 257–259.
33. Rapraeger, A., Krufka, A. & Olwin, B. (1991) *Science* **252**, 1705–1708.
34. Yayon, A., Klagsbrun, M., Esko, J. D., Leder, P. & Ornitz, D. M. (1991) *Cell* **64**, 841–848.
35. Moscatelli, D. (1988) *J. Cell Biol.* **107**, 753–759.
36. Baird, A., Schubert, D., Ling, N. & Guillemin, R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2324–2328.
37. Moscatelli, D. & Devesly, P. (1990) *Growth Factors* **3**, 25–33.
38. Nugent, M. A. & Edelman, E. R. (1992) *Biochemistry* **31**, 8876–8883.
39. Nugent, M. A. & Edelman, E. R. (1992) *J. Biol. Chem.* **267**, 21256–21264.
40. Ishai-Michaeli, R., Svahn, C. M., Weber, M., Chajek-Shaul, T., Korner, G., Ekre, H.-P. & Vlodavsky, I. (1992) *Biochemistry* **31**, 2080–2088.
41. Hay, E. (1991) *Cell Biology of the Extracellular Matrix* (Plenum, New York).
42. Vlodavsky, I., Folkman, J., Sullivan, R., Fridman, R., Ishai-Michaeli, R., Sasse, J. & Klagsbrun, M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2292–2296.
43. Folkman, J., Klagsbrun, M., Sasse, J., Wadzinski, M., Ingber, D. & Vlodavsky, I. (1988) *Am. J. Pathol.* **130**, 393–400.
44. Barger, A. C., Beeuwkes, R., III, Lainey, L. L. & Silverman, K. J. (1984) *N. Engl. J. Med.* **310**, 175–177.
45. Cuevas, P., Gonzalez, A. M., Carceller, F. & Baird, A. (1991) *Circ. Res.* **69**, 360–369.
46. Whalen, G. F., Shing, Y. & Folkman, J. (1989) *Growth Factors* **1**, 157–164.