Distribution of intravenously administered acidic and basic fibroblast growth factors in the mouse

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Summary. Iodinated acidic or basic fibroblast growth factor (aFGF or bFGF) were separately injected into adult mice to follow their distribution in the main organs of the animals. Iodinated FGFs intravenously injected into mice cleared from blood with a $T_{1/2}$ of 30 s. They mainly bound to kidney, liver and spleen. The binding of FGFs to these organs was maintained when the latter were washed with a physiological buffer containing 0.15 M NaCl, but it was eliminated when the buffer contained 2 M NaCl. Simultaneous injections of the FGFs together with increasing doses of heparin weakened the binding of FGF to vessels in a dose-dependent manner.

Key words. aFGF and bFGF; blood circulation; mouse.

Acidic and basic fibroblast growth factors (a and bFGF) are closely related peptides. Amino acid sequence analyses identified a 55% homology between these growth factors, which share similar biological properties[1]. In vitro, a and bFGF are mitogens for various mesoderm and neuroectoderm derived cells like fibroblasts, endothelial cells, smooth muscle cells and glial cells[2]. In addition, they can induce differentiation and the outgrowth of neurites in neurons from various origins[2]. FGFs seem to mediate their biological effects via cellular membrane receptors and can be stored and released from heparin-like structures present in the extracellular matrix[3]. In vivo, FGFs are ubiquitously distributed in normal, developing and pathological tissues[3]. Although the in vitro properties and the in vivo distribution suggest a role for these growth factors in embryonic development and in tissue homeostasis, their true physiological function remains unknown[2]. Nevertheless, it has been shown that FGFs are potent angiogenic molecules[4] and could thus be of therapeutic interest in vascular diseases[5]. In order to approach an in vivo use of these growth factors, we have studied their distribution in the mouse tissues after their intravenous injection.

Materials and methods

Two-month-old Swiss mice from our laboratory colony were housed under the usual conditions. Acidic and basic FGFs were purified from bovine brain[6] and radioiodinated as previously described[7]. Iodinated FGF specific activity was 100,000 cpm/ng. Heparin was obtained from Sigma.

Mice were anesthetized by a 5-mg i.p. injection of ketamine base in aqueous solution (10 mg/ml) (Mérieux). The abdominal cavity was opened and 1 ng of [^{125}I] a or bFGF in 10 µl of 10 mM phosphate buffer, pH 7.4 was injected into the inferior vena cava using a 25 µl Hamilton syringe. At different times after injection (0.5, 1, 2, 5, 8, 10 min), the animals were bled by removing one eye. Several organs (liver, kidneys, spleen, lungs, heart, stomach, intestine, brain and gonads) were separately collected and weighed, and the radioactivity of these whole organs was measured with a Gamma counter. A second group of mice was treated as described above, but 5 min after the FGF injection, 20 ml of phosphate buffered saline containing either 0.15 or 2 M NaCl was perfused into the aorta for 10 min before the killing of the animals and organ analysis. In a third group, simultaneous injections of iodinated FGFs and different doses of heparin (0.25, 2.5, 25 ng) were performed. Five minutes later, their blood and organs were collected and analyzed.

Results

Iodinated acidic or basic FGF rapidly cleared from the blood (fig. 1). Thirty seconds after radiolabeled FGF injection in mice, the radioactivity value in the blood was reduced by half. One minute and more after injection, radioactivity was no longer detected in the blood. In parallel, the full radioactivity was detected in organs. The radiolabeled FGFs mainly bound to three out of the nine studied organs, namely kidney, liver and spleen (table). The results from the second series showed that the binding of FGF to organs was maintained after a washing with a buffer containing 0.15 M NaCl, but 90% of this binding disappeared after a washing with a buffer con-

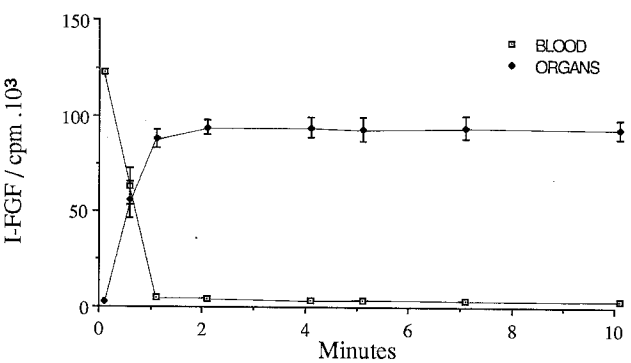

Figure 1. Time course of [^{125}I] a and bFGF binding in organs after intravascular injection. One ng of [^{125}I] FGF was perfused into the inferior vena cava. Each point shows the means ± SE for 6 animals.

Distribution of [^{125}I] a and bFGF in organs after their intravascular injection. One ng of [^{125}I] FGF was perfused into the inferior vena cava. Five minutes later, different organs were cut off and their radioactivity was measured. The values are in cpm/g of fresh tissue.

Organs	^{125}I-acidic FGF binding (cpm/g of tissue)	^{125}I-basic FGF binding (cpm/g of tissue)
Liver	14900 ± 3980	18750 ± 1240
Kidneys	26430 ± 2100	20700 ± 5150
Spleen	3840 ± 990	4120 ± 830
Brain	60 ± 25	70 ± 30
Heart	330 ± 70	380 ± 90
Lung	490 ± 200	500 ± 230
Stomach	390 ± 150	250 ± 190
Intestine	650 ± 700	920 ± 800
Gonads	1360 ± 1280	790 ± 830

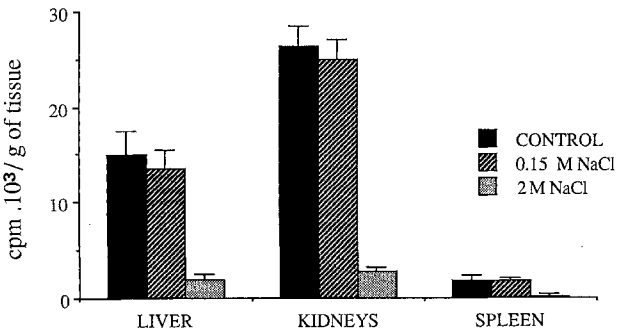

Figure 2. Effect of NaCl concentration on FGF binding. After injection of FGF, as described in Materials and Methods, the mice were perfused with 20 ml of 10 mM phosphate buffer (pH 7.4) containing either 0.15 M NaCl or 1 M NaCl.

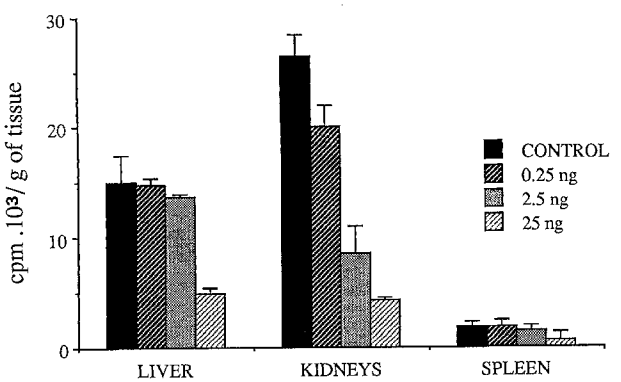

Figure 3. Effects of heparin concentration on FGF binding. [^{125}I] a or bFGF were injected together with bolus of heparin.

taining 2 M NaCl (fig. 2). Finally, the radioactivity in the organs of the third series of mice, which were injected with labeled a or bFGF together with increasing amounts of heparin, was reduced in a heparin dose-dependent manner, indicating a decrease of the binding of these factors (fig. 3).

Discussion

Both aFGF and bFGF behaved similarly after their intravenous injection in mice. The injected FGFs in the blood were cleared with a $T_{1/2}$ of 30 s and appeared at the same time in the organs. Radioautographic studies of these organs indicated that FGFs were bound to the blood vessels (data not shown). FGFs are known to bind to membrane receptors and to heparin-like molecules that are present on the cell surface and in the extracellular matrix [8]. Since the binding of FGFs to the membrane receptors was sensitive to 2 M NaCl [8], our results suggest that the iodinated FGFs bind mainly to heparin-like molecule at the surface of the blood vessels. This interpretation is reinforced by our results concerning injection of heparin, which showed that the FGF binding was easily displaced by heparin, and by the results obtained by radioreceptor assay on an isolated vessel [9]. The special binding of FGFs to a few organs might be explained by their heavy vascular system. Nevertheless, we also showed that richly vascularized organs close to the injection site, like heart and lung, bound only a small amount of FGF. Thus the binding capacity of FGF to vessels, which is different from one organ to another, appears to be related to the presence or absence of competent heparin-like molecules. One question is whether exogenous injected FGF remains bioavailable, since the results of this report showed that injected FGF was probably rapidly sequestered by a heparin-like molecule. FGF could be released to stimulate endothelial cell nucleus DNA synthesis [10]. Now, the conditions of in vivo releasing and utilization of FGFs remain to be investigated.

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