Infused IGF-I/IGFBP-3 complex causes glomerular localization of IGF-I in the rat kidney

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Sandra, Alexander, Mary Boes, Brian L. Dake, John B. Stokes, and Robert S. Bar. Infused IGF-I/IGFBP-3 complex causes glomerular localization of IGF-I in the rat kidney. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E32–E37, 1998.—Insulin-like growth factor I (IGF-I) increases renal blood flow, glomerular filtration rate (GFR), and proximal tubule reabsorption of phosphate in humans and rodents. The biological effects of IGF-I are likely to be influenced by cellular localization of IGF-I within the kidney. We therefore tested whether the renal localization of infused IGF-I could be altered if given with selected IGF-binding proteins (IGFBPs). Rats were treated with intravenous injections of IGF-I, IGF-I/IGFBP-3, IGF-I/IGFBP-4, or IGFBP-4 alone or with complexes of IGF-I/IGFBP-3 or IGFBP-4. The cellular localization of IGF-I and IGFBP within the kidney was determined. IGF-I and IGFBP-3 complexes were found almost exclusively in proximal renal tubules. In contrast, about one-third of IGF-I/IGFBP-3 and one-half of IGF-I/IGFBP-4 was localized to glomeruli. When IGF-I was given alone, 3% was found in glomeruli and 89% in proximal tubules. When given as IGF-I/IGFBP-3, 29% was in glomeruli and 65% in proximal tubules. We conclude that the cellular localization of IGF-I within the kidney can be directed to glomerular elements if the IGF-I is given with IGFBP-3.

In the intact animal, perhaps the most IGF-sensitive tissue/organ is the kidney. In both rodents and humans, treatment with relatively low doses of IGF-I causes a rapid increase in renal plasma flow and glomerular filtration rate (14–16). To gain insight into the potential influence of IGFBP-3 on IGF-mediated renal function, in the present study rats were infused with IGF-I alone, IGFBP-3 alone, or IGF-I plus IGFBP-3, and the tissue localization within the kidney was determined by autoradiographic methods. Localization data for infused IGFBP-3 were then compared with the tissue localization data in the kidney of a second group of animals perfused with IGFBP-4 or IGFBP-4/IGF-I complexes. As previously reported by Arany et al. (2), IGFBP-3 was found throughout the kidney, with particular localization in cellular elements of the glomerulus. In contrast, IGF-I, as well as IGFBP-4, was found almost exclusively in proximal renal tubules, with striking enrichment in vacuolar structures of the tubules. When IGF-I was infused with IGFBP-3, the localization of IGF-I was more closely approximated that of IGFBP-3, with an increase of IGF-I in the glomerulus and a decrease in localization within the proximal tubules. These data indicate that the kidney localization, and perhaps renal bioactivity, of infused IGF-I can be altered if the IGF-I is infused in the presence of IGFBP-3.

MATERIALS AND METHODS
IGF-I (Intergen, Purchase, NY), IGFBP-3 (a gift from Celtrix, Santa Clara, CA), and IGFBP-4 (made in Baculovirus as per protocol of Invitrogen (San Diego, CA)) were iodinated with NaI21 by the lactoperoxidase method as previously described (13). Specific activities of IGF-I, IGFBP-3, and IGFBP-4 ranged from 40 to 100 µCi/µg protein. Heart perfusion buffer (HPB) consisted of Hanks’ balanced salt solution, pH 7.4, with glucose (1 g/l), fatty acid-poor albumin (1 mg/ml; Intergen), and 1 mM HEPES (Sigma Chemical, St. Louis, MO) in place of sodium bicarbonate.

Perfusion Studies
Sprague-Dawley rats, 250–274 g (Harlan Laboratories, Indianapolis, IN), were anesthetized with methoxyflurane (Pitman-Moore, Mundelein, IL) before tail vein injections with IGF-I and/or IGFBPs. IGF-I, IGFBP-3, and IGFBP-4 were each injected into the tail vein and allowed to circulate for 1 or 10 min. The chest cavity of the animal was then opened, a 19-gauge needle was inserted into the left ventricle, and 200 µl of blood were removed for analysis of degradation of the labeled protein. To clear blood from the circulation, the rat was next perfused through the ventricular needle with 100 ml of PBS, 37°C, at 130 mmHg by gravity flow. To fix

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tissues, the perfusate was then changed to a solution of 2.5% glutaraldehyde in 67 mM sodium phosphate, and 100 ml of the fixing solution were infused.

Rats were also treated with either IGFBP-3 and 125I-IGF-I or IGFBP-4 and IGF-I. Treatment of an animal with both IGFBP and IGF-I was done in two ways. 1) Rats were preperfused with unlabeled IGFBP by injecting 100 µg of unlabeled IGFBP (IGFBP-3 or IGFBP-4) in 300 µl HPB, a mixture that was allowed to circulate for 10 min. The rat was then given 2 × 10^7 cpm 125I-IGF-I in 300 µl HPB; this was allowed to circulate for 1 min, after which the kidney tissues were fixed and analyzed. 2) Rats were infused with a mixture of 125I-IGF-I (2 × 10^7 cpm) and unlabeled IGFBP-3 or IGFBP-4 in a 1:10 IGF-I/IGFBP molar ratio. After 1 min, the tissues were fixed and prepared for analysis.

Tissue Fixation

Light-microscopic level autoradiography. Kidneys were rapidly dissected and cleaned of perirenal fat and connective tissue. The kidneys were then cut coronally, processed by standard methods, and embedded in paraffin. Sections (8 µm) were cut, mounted on slides, deparaffinized, and coated with Kodak NTB2 nuclear track emulsion. After 1–2 wk of development, the slides were processed with D-19 developer and Kodak NTB2 nuclear track emulsion. After 1–2 wk of development, the slides were stained with hematoxylin and eosin and were analyzed (as described in Analysis) for silver grain distribution with a light microscope equipped with a ×60 and a ×100 objective lens.

Electron-microscopic level autoradiography. Rats were injected as described for light-microscopic level autoradiography except that the counts per minute of the injected IGF-I or binding protein were increased 10-fold. Following removal of the kidneys, representative cortical and medullary areas were dissected out and processed for electron microscopy by postfixing in osmium tetroxide, dehydration in graded ethanol, and embedding in Epoxy resin. Thin (50 nm) sections of the tissue in the areas of the glomerulus and tubules were cut, mounted on nickel grids, and covered with a monolayer of Ilford L-5 emulsion. After 2–4 wk of development, the grids were processed using Microdol-x developer. Grids were stained with lead citrate and uranyl acetate before being viewed in a Hitachi E600 electron microscope.

Analysis

Silver grains at both the light- and electron-microscopic levels were counted and analyzed for localization. For 125I-IGF-I alone, 125I-IGFBP-3 alone, and IGFBP-3 plus 125I-IGF-I, the data represent the distribution of ~60,000 silver grains counted at the light level in at least three independent experiments. An equal number of microscopic fields were randomly chosen and analyzed in each treatment group, and individual silver grains were assigned to a cellular compartment. The cellular compartment assigned was based on the localization of the grains to a distance within 0.2 µm. The density of background silver grains on the periphery of each slide was subtracted from the density over tissue sections. This background averaged 5% of the grain density associated with cells. Values are means ± SE of at least five rat kidneys. For studies with IGFBP-4, both kidneys from a single rat were analyzed.

RESULTS

Rats were infused with 125I-IGF-I, 125I-IGFBP-3, or 125I-IGFBP-4. The relative distribution of radioactivity in several organs/tissue is given in Table 1. After 1-min circulation of the 125I-labeled protein, the kidneys were removed and analyzed for 125I grains at the light- and electron-microscopic levels. 125I-IGF-I (with or without IGFBP-3 or IGFBP-4) was infused into the tail vein of the rat. For tissues, cpm/g, and for blood, cpm/ml are compared. Kidney radioactivity was assigned a cpm value of 1, with radioactivity of other tissues and organs given values relative to kidney cpm. Similar results were obtained in 4 and 5 separate experiments for 125I-IGFBP-3 and 125I-IGFBP-4, respectively.

Table 1. Distribution of infused 125I-IGFBP-3, 125I-IGFBP-4, and 125I-IGF-I

<table>
<thead>
<tr>
<th>Tissue/Organ</th>
<th>125I-IGFBP-3</th>
<th>125I-IGFBP-4</th>
<th>125I-IGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.06</td>
<td>0.04</td>
<td>0.38</td>
</tr>
<tr>
<td>Liver</td>
<td>2.7</td>
<td>1.08</td>
<td>0.38</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.0</td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Lung</td>
<td>1.2</td>
<td>0.1</td>
<td>0.59</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.3</td>
<td>0.28</td>
<td>0.52</td>
</tr>
<tr>
<td>Bone</td>
<td>0.2</td>
<td>0.09</td>
<td>0.66</td>
</tr>
<tr>
<td>Blood</td>
<td>1.0</td>
<td>1.1</td>
<td>5.9</td>
</tr>
</tbody>
</table>

Approximately 20 × 10^6 counts/min (cpm) of 125I-labeled insulin-like growth factor-binding protein-3 (IGFBP-3), 125I-IGFBP-4, or 125I-IGF-I were injected into the tail vein of the rat. For tissues, cpm/g, and for blood, cpm/ml are compared. Kidney radioactivity was assigned a cpm value of 1, with radioactivity of other tissues and organs given values relative to kidney cpm. Similar results were obtained in 4 and 5 separate experiments for 125I-IGFBP-3 and 125I-IGFBP-4, respectively.

IGFBP-4 and IGF-I Perfusions

At the light-microscopic level, autoradiographic grain counts demonstrated that infused 125I-IGF-I and 125I-IGFBP-4 were each localized to the proximal tubule, with a glomerulus-to-proximal tubule ratio of ~1:30 (Fig. 1, Table 2). When 125I-IGF-I was infused with unlabeled IGFBP-4 after IGFBP-4 pretreatment, or chemically cross-linked to IGFBP-4 (data not shown), the distribution of 125I-IGF-I within the kidney was unchanged (Table 2). With higher magnification at the light level, the majority of the silver grains for both 125I-IGF-I and 125I-IGFBP-4 appeared localized over vacuoles near the apical surface of the tubule epithelium (Fig. 2). Electron microscopy revealed that intracellular silver grains representing IGF-I or IGFBP-4 were predominantly localized to vacuolar structures.
most likely early endosomes resulting from endocytotic activity (Fig. 3). After 1 and 10 min of circulation, 

\[ ^{125}\text{I}-\text{IGF-I} \] and \[ ^{125}\text{I}-\text{IGFBP-4} \] in the blood were >90% intact, as judged by TCA precipitation or SDS-PAGE analysis.

**IGFBP-3**

In contrast to IGFBP-4, the renal distribution of infused \[ ^{125}\text{I}-\text{IGFBP-3} \] was shifted to the glomerulus with a glomerulus-to-proximal tubule ratio of ~2:3, i.e., 39% of the IGFBP-3 grains were in glomeruli, 57% in proximal tubules, and 3 and 1% in distal tubules and collecting ducts, respectively (Fig. 4, Table 2). Electron microscopy of the glomerulus revealed that 55% of the silver grains were associated with podocytes or podocyte processes. The remaining grains within the glomeruli were distributed between the capillary endothelium/basement membrane (30%) and mesangium (15%) (Fig. 5).

\[ ^{125}\text{I}-\text{IGF-I} \] was also infused in IGFBP-3-treated rats. This was done in two ways. 1) Rats were first preperfused with IGFBP-3 (100 µg injected in tail vein). The unlabeled IGFBP-3 was allowed to circulate for 10 min, and then \[ ^{125}\text{I}-\text{IGF-I} \] (2 x 10^7 cpm in 300 µl buffer) was injected in the tail vein and allowed to circulate for 1 min. 2) \[ ^{125}\text{I}-\text{IGF-I} \] (2 x 10^7 cpm) and unlabeled IGFBP-3 in a 1:10 ratio were coinfused and allowed to circulate for 1 min. Both treatments resulted in a changed renal distribution of \[ ^{125}\text{I}-\text{IGF-I} \] (Table 1). In rats preperfused with IGFBP-3, 8% of grains were in the glomeruli and 77% were in the proximal tubule. In rats in which \[ ^{125}\text{I}-\text{IGF-I} \] was coinfused with IGFBP-3, the redistribution of \[ ^{125}\text{I}-\text{IGF-I} \] to glomerular structures was more striking, with 29% of grains localized to the glomerulus and 65% to the proximal tubule, a distribution similar to that of rats treated with \[ ^{125}\text{I}-\text{IGFBP-3} \] alone (39% glomeruli, 57% proximal tubule) and clearly different from renal localization of \[ ^{125}\text{I}-\text{IGF-I} \] in rats treated solely with \[ ^{125}\text{I}-\text{IGF-I} \] (3% glomeruli, 89% proximal tubule). After 1 min of circulation, >90% of \[ ^{125}\text{I}-\text{IGF-I} \] and \[ ^{125}\text{I}-\text{IGFBP-3} \] in the blood represented intact protein, as judged by TCA precipitation or SDS-PAGE analysis.

**DISCUSSION**

In the present study, \[ ^{125}\text{I}-\text{IGF-I} \], \[ ^{125}\text{I}-\text{IGFBP-4} \], and \[ ^{125}\text{I}-\text{IGFBP-3} \] were infused into rats, and the distribution of the labeled proteins within the kidney was determined. In addition, the effect of each binding protein on the renal localization of infused \[ ^{125}\text{I}-\text{IGF-I} \] was also determined. Infused IGF-I, IGFBP-4, and
IGFBP-4 with IGF-I were localized in endocytotic vacuoles of the proximal tubules, the major degradative pathway in the kidney. In contrast, for infused IGFBP-3, nearly 40% of the renal IGFBP-3 was retained in glomeruli, associated with podocytes, endothelium/basement membrane, and mesangial cells. Of perhaps greatest interest, the localization of infused IGF-I within the kidney was substantially altered when IGF-I was introduced after IGFBP-3 pretreatment or, more strikingly, if IGF-I and IGFBP-3 were infused.
together. When given as an IGF-I/IGFBP-3 coinfusion, 29% of renal IGF-I was in glomeruli vs. only 3% of IGF-I in the glomeruli when IGF-I was injected alone.

In both humans and rats, treatment with IGF-I results in a rapid increase in glomerular filtration rate (GFR), renal blood flow, and proximal tubular reabsorption of phosphate. The precise cellular mechanisms by which IGF-I mediates these effects are not entirely clear. Type I IGF (IGF-I) receptors, as well as the ability to synthesize IGF-I, are both properties of the three cell types of the glomerulus (endothelial, mesangial, and epithelial cells) (3, 12). In contrast, the proximal tubule has a high density of IGF-I receptors but little ability to synthesize IGF-I, whereas, in humans, the cells of the distal tubule and collecting ducts synthesize substantial IGF-I but lack specific IGF-I receptors. The mismatch of IGF-I receptors and endogenous IGF-I synthesis along the nephron has suggested the potential for autocrine, paracrine, and endocrine functions of IGF-I within the kidney (17). Furthermore, it may be reasonable to speculate that the IGF-I-induced increase in renal plasma flow and perhaps in GFR is likely mediated through the endothelial and mesangial cells of the glomerulus, whereas the increase in phosphate reabsorption is initiated through IGF-I interaction with the dense concentration of type I IGF receptors in the proximal tubule, the major site of phosphate reabsorption in the kidney (9).

Further impacting on the results of the present study is the finding that, in rats and perhaps humans, the kidney is the major site of degradation of injected or infused IGF-I. This occurs despite the presence of increased receptors for IGF-I in other tissues, e.g., skeletal muscle and heart, as well as the presence of enzymes capable of degrading IGF-I in most tissues of the rat. In a recent report, Tanaka et al. (30) injected rats with recombinant human IGF-I and determined localization and degradation of IGF-I in tissues 15 and 60 min after injection. The kidney was the only tissue having a higher concentration of IGF-I than plasma and accounted for 69% of the elimination of the injected IGF-I from the circulation. Therefore, it is not unreasonable to speculate that altering the localization of injected IGF-I within the kidney by giving it with IGFBP-3 could potentially alter the effects of exogenously administered IGF-I.

In recent years, several clinical trials of IGF-I therapy have suggested beneficial effects of IGF-I in disorders as diverse as growth hormone insensitivity (Laron dwarfism) (22), renal failure (26), insulin resistance syndromes and diabetes mellitus (21, 29), AIDS (23), and osteoporosis (19). In these clinical studies it was determined that the frequency and severity of side effects progressively worsened as the dose of IGF-I was increased, often limiting the amount of IGF-I that could be given. Thus, at subcutaneous IGF-I doses >160 μg·kg−1·day−1, chronic IGF-I therapy became difficult because of side effects. In attempts to diminish IGF-I side effects while maintaining its beneficial actions, a limited number of studies have reported successfully giving IGF-I to humans as IGF-I/IGFBP-3 complexes (1). An additional rationale for giving IGF-I as a complex with IGFBP-3 is based on the finding that when IGF-I and IGFBP-3 in the circulation are present as part of the 150-kDa complex (IGFBP-3/IGF-I/acid labile subunit), the IGF-I and IGFBP-3 are largely limited to the vascular compartment, having plasma half-lives of ~16 h (2, 3, 17). However, when IGF-I is in the ~50-kDa IGF-I/IGFBP-3 complex, the complex readily traverses the capillary endothelium, having a plasma half-life of ~25 min. The findings of the present study suggest an additional potential renal benefit of combined IGF-I/IGFBP-3 therapy. When IGF-I is given in this complex, not only could higher doses of IGF-I be given to humans, but the potential diversion of IGF-I away from degradative pathways in the kidney could potentiate the renal effects of IGF-I. Studies in humans comparing the renal effects of therapy with IGF-I alone or IGF-I given as IGF-I/IGFBP-3 complex are required to corroborate or refute this speculation.

This work was supported by research funds from the Veterans Administration and National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-25421, DK-25295, and DK-52617.

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Received 23 Jan 1998; accepted in final form 3 April 1998.

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