The Insulin-like Growth Factors (IGFs) I and II Bind to Articular Cartilage via the IGF-binding Proteins*

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Bovine articular cartilage discs (3 mm diameter × 400 μm thick) were equilibrated in buffer containing 125I-insulin-like growth factor (IGF)-I (4 °C) ± unlabeled IGF-I or IGF-II. Competition for binding to cartilage discs by each unlabeled IGF was concentration-dependent, with ED50 values for inhibition of 125I-IGF-I binding of 11 and 10 nM for IGF-I and -II, respectively, and saturation by 50 nM. By contrast, an analog of IGF-I with very low affinity for the insulin-like growth factor-binding proteins (IGF-BPs), des-(1–3)-IGF-I, was not competitive with 125I-IGF-I for cartilage binding even at 100–400 nM. Binding of the 125I-labeled IGF-II isoform to cartilage was competed for by unlabeled IGF-I or -II, with ED50s of 160 and 8 nM, respectively. This probably reflected the differential affinities of the endogenous IGF-BPs (IGF-BP-6 and -2) for IGF-II/IGF-I. Transport of 125I-IGF-I was also measured in an apparatus that allows diffusion only across the discs (400 μm), by addition to one side and continuous monitoring of efflux on the other side. The time lag for transport of 125I-IGF-I was 268 min, an order of magnitude longer than the theoretical prediction for free diffusion in the matrix. 125I-IGF-I transport then reached a steady state rate (% efflux of total added 125I-IGF/unit time), which was subsequently accelerated ~2-fold by addition of an excess of unlabeled IGF-I. Taken together, these results indicate that IGF binding to cartilage, mostly through the IGF-BPs, regulates the transport of IGFs in articular cartilage, probably contributing to the control of their paracrine activities.

Insulin-like growth factors (IGFs) are key regulators of matrix homeostasis in articular cartilage (1, 2). During osteoarthritis (OA), the metabolic balance is lost in favor of degradation, and it has been suggested that this may be due to an insensitivity of resident chondrocytes to IGF-I stimulation (3). Recent studies have sought to understand the underlying mechanisms, and emphasis has been placed on understanding the role of the IGF-binding proteins (IGF-BPs). This group of proteins has the ability to modulate the actions of the IGFs, either enhancing or inhibiting them, depending largely on their post-translational modifications and tissue localization (for reviews, please refer to Refs. 4 and 5). An increase in IGF-BP mRNAs in osteoarthritic compared with normal chondrocytes has been observed (3, 6), and this increased expression is accompanied by increased IGF-BP proteins in the culture medium of OA cartilage slices (6) or in monolayer cultures (3, 7). There are also reports of increased IGF-I (6, 8) and IGF type I receptor (7) in OA compared with normal articular cartilage. These findings are reconciled by the proposal that the IGF-BPs play an inhibitory role during the disease, blocking the actions of excess IGF-I (3, 6, 7). While the function of the IGF-BPs in osteoarthritic cartilage remains to be proven, the role of IGF-BPs in normal articular cartilage was recently studied in bovine cartilage organ cultures. These experiments compared the action of native IGF-I to that of site-mutated analogs of IGF-I with very low binding affinity for IGF-BPs, but with nearly normal binding to the signaling receptors (10). The IGF-I analogs were more effective than their native counterpart in stimulating proteoglycan synthesis when added to cultured cartilage slices, strongly suggesting that the dominant endogenous IGF-BP activity was inhibitory to IGF action.

Synovial fluid has a significant concentration of IGFs, ~20–50 ng/ml in adult normal human (9, 11), and may provide a significant source of IGFs to cartilage. Chondrocytes express IGF-I mRNA and may also contribute to the endogenous pool of this growth factor (6, 8). In adult articular cartilage, single chondrocytes are surrounded by vast areas of matrix, through which IGFs must be transported to reach a responsive cell. A general proposal in the literature is that transport of IGFs from the circulation to extravascular spaces may be controlled by the IGF-BPs; this may be a mechanism governing paracrine activities of IGFs within tissues as well. Studies that directly examine binding of IGFs to intact tissue and/or their transport have been very sparse and have not been fully comprehensive. To enhance our understanding of the interactions of the IGFs with endogenous tissue sites and the diffusion of this growth factor through cartilage, we now quantify the binding of IGFs to bovine articular cartilage, and examine the involvement of IGF-BPs in this process. We provide a line of evidence that IGFs bind to articular cartilage discs through IGF-BPs. 1) Binding of 125I-IGF-I or II to cartilage is specific; 2) an analog of IGF-I (des-(1–3)-IGF-I) with very low affinity for the IGF-BPs but normal affinity for the signaling receptor is unable to compete with radiolabeled IGF-I for binding to the cartilage discs, while the native IGF is a highly effective competitor; and 3) the characteristics of 125I-IGF-I and II binding to the tissue

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§ The abbreviations used are: IGF, insulin-like growth factor; IGF-BP, IGF-binding protein; Chaps, 3-[3-cholamidopropyl]dimethylammonio]propanesulfonic acid; OA, osteoarthritis; PBS, phosphate-buffered saline; BSA, bovine serum albumin; o-Phe, orthophenanthroline; MCP, metacarboxylase; FPG, femoropatellar groove.

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are as expected for their binding in solution to IGF-BP-6, a major bovine cartilage IGF-BP. In addition, we use a transport apparatus to show directly that IGF binding contributes to the regulation of radiolabeled IGF transport through cartilage discs. Our studies provide strong support for the concept that IGF-BPs are important regulators of the bioavailability of IGFs in articular cartilage.

**EXPERIMENTAL PROCEDURES**

**Materials**

Human recombinant (hr) 3-[^125]Iiodotyrosyl-IGF-I and II were obtained from Amersham Pharmacia Biotech. Bovine serum albumin (BSA) was radioimmunoassay grade, from Sigma. hrIGF-II and II were obtained from B&H Systems, Inc., or from Gropep Pty Ltd (Adelaide, Australia); hrIGF-I from the latter source was used for studies as indicated in the text. Des-(1–3)-IGF-I was also from Gropep. The hrIGF-BP-2 and hrIGF-BP-4 standards were from Austral Biologicals.

**Buffers**

- **Phosphate Buffer**—Phosphate buffer consisting of 0.03 M NaH₂PO₄, pH 4.4, containing 0.02% sodium azide and 0.1% BSA.
- **Binding Buffer**—Binding buffer consisted of phosphate-buffered saline (PBS), pH 7.4, containing 0.1% BSA and protease inhibitors (5.6 mM E-64, 1 mM Pefabloc SC, 0.7 µg/ml peptatin A, and 1 mM orthophenanthroline (o-Phe).
- **Urea Buffer**—Urea buffer consisted of 0.05 M Tris maleate, pH 6.0, containing 8 M urea, 0.3 M NaCl, 0.05% Chaps, 5 mM phenylmethylsulfonyl fluoride, 3 mM o-Phe, and 4.5 µg/ml each of peptatin A and leupeptin.
- **Acid Dialysis Solution**—Acid dialysis solution consisted of 4 mM HCl, containing 1 mM o-Phe, 0.1 mM phenylmethylsulfonyl fluoride, and 0.1 µg/ml each of peptatin A and leupeptin.

**General Procedures**

**Chromatography and Concentration of Radiolabeled IGFS**—Immediately prior to use, the lyophilized[^125]I-IGF was dissolved in 300 µl of 0.01 M Hac + 0.1% BSA, and run on a 0.6 × 30-cm Sephadex G50 column equilibrated in phosphate buffer at 14 °C to ensure removal of any small molecular weight radiolabel. The fractions corresponding to authentic unlabeled hrIGF standard were pooled, concentrated, and exchanged into binding buffer in a Centricon-3 filter (Amicon).

**Extraction and Isolation of IGF-BPs from Bovine Articular Cartilages**—This was done essentially as described before (10). Briefly, slices of bovine articular cartilage from the femoropatellar grooves of adult animals (of approximately 18–24 months of age), or from the metacarpophalangeal joints of 2–6-week-old calves were diced into small pieces and extracted in urea buffer at 4 °C. The extracts were run on DEAE-Sephadex equilibrated in the same buffer to remove proteoglycans (which bind to the column), the effluent fractions were pooled, dialyzed against acidic solution, and concentrated in a speed vacuum centrifuge. Glycosaminoglycan content in the samples was determined by the dimethylmethylen blue dye binding procedure (12), and protein was analyzed by the bicinchoninic procedure (MicroBCA), as directed by the manufacturer (Pierce).

**Western Ligand Blotting for IGF-BPs**—The dried samples were resuspended directly in non-reducing SDS sample buffer at 1 µg of protein/µl, and 25 µg applied per electrophoresis well. Western ligand blots were carried out as described previously using[^125]I-IGF-II as the binding ligand (13).

**Cartilage Disc Preparation**

Cylindrical plugs of cartilage with the underlying bone slightly greater than 9 mm in diameter were cored from the femoropatellar grooves of 18–24-month-old steers. Cartilage-bone plugs were clamped in a slide microtome and the top ~150 µm of superficial cartilage was removed. Plane parallel discs of 400 µm thickness were then obtained and subsequently punched to 3 or 9 mm diameter. During the harvest, tissue was maintained hydrated with PBS supplemented with 2 mM EDTA, penicillin (100 units/ml), streptomycin (0.1 mg/ml), and amphotericin B (0.25 µg/ml).

**Equilibrium Binding of IGFS to Bovine Articular Cartilage**

Adult bovine articular cartilage (3 mm diameter × 400 µm thick) was prepared from femoropatellar groove cartilage as described above. After a 10–20-h equilibration in binding buffer, the discs were transferred to 24-well culture plates containing fresh buffer. In an effort to randomize any differences between different parts of the joint, four to five discs were carefully selected, one from each from different anatomical locations, and pooled in each well.[^125]I-IGF-I or II, as appropriate, was then added to each well (specific activity 2000 Ci/mmol, an average of 2 × 10⁶ cpm in 10 μl[^125]I-IGF-I, or 0.053 μM[^125]I-IGF-II). Graded levels of unlabeled hrIGF-I, hrIGF-II, or des-(1–3)-IGF-I were then added (or no additions made) as indicated for each experiment. The incubation volume of 1.5 ml/well was at least 100 times the cartilage volume. Following a 48-h incubation at 4 °C, each disc was transferred into a wash well containing fresh, IGF-free binding buffer, removed within seconds, and transferred into a counting vial. The equilibrium fluid for each well was counted sequentially. Immediately prior to weighing, each disc was blotted dry on gauze pads. In selected experiments, the equilibrium fluid was analyzed by Sephadex G-50 chromatography to determine whether there had been significant degradation of the[^125]I-IGF during the incubation. This was not the case, as >95% of the radiolabel comigrated with the authentic unlabeled IGF standard (results not shown).

**Diffusive Transport Experiments**

Cartilage discs (9 mm diameter × 400 µm depth) were maintained at 4 °C in PBS with inhibitors (as described above) until mounted in the transport chamber. The transport measurement system has been previously described in detail (14). Briefly, a two-compartment acrylic diffusion chamber (Fig. 5, inset) had ports to fit up to five cartilage discs (exposed tissue area for transport = 0.36 cm²/disc). Gaskets and O-rings were used to seal the tissue samples in place so that, when the two chamber halves were assembled, transport between compartments could occur only through the tissue. The baths on either side of the cartilage consisted of PBS supplemented with protease inhibitors (2 mM EDTA, 5 mM benzamidine HCl, and 1 mM phenylmethylsulfonyl fluoride). Temperature was maintained at 20 °C. Both compartments were open to ambient pressure, and portions of the downstream bath were recirculated at 1–3 ml/min through a modified flow-through 125I-radioactivity detector (14). Downstream radioactivity as measured by the detector was continuously recorded at 6-s intervals by the computer.

**RESULTS**

**Identification of Major IGF-BPs in Bovine Articular Cartilage from the Femoropatellar Groove of Adult Animals**—Previous work (10) identified IGF-BPs in cartilage obtained from the MCP joints of young and adult bovines. The MCP cartilage is relatively thin and rests on curved bone surfaces. To facilitate the preparation of cartilage discs of 400 µm depth, we used femoropatellar groove (FGP) cartilage from adult animals, which is relatively thick and rests on a fairly smooth planar surface. Biochemical variations in cartilages from different joints, including differential susceptibility to metabolic effectors have been reported (15), so it was important to determine if the bovine FPG cartilage had a similar IGF-BP profile as the previously characterized tissue dissected from MCP joints. To provide a direct side by side comparison, cartilage slices from each of the two joint sources (FGP and MCP) were each extracted, purified, and analyzed under parallel conditions (see "Experimental Procedures"). Fig. 1 shows the Western ligand blot.[^125]I-IGF-II was used as the binding ligand since it detects all the IGF-BPs at least as well as the IGF-1 ligand (13), and ensures detection of IGF-BP-6, which reacts weakly if at all with[^125]I-IGF-I under these conditions (10, 13). Lane 1 shows the migration of IGF-BPs from the MCP cartilage, and lanes 2 and 3 show the binding proteins from FGP cartilage derived from the joints of two different bovines. As can be seen, the major IGF-BP in all three lanes migrated to a position above that of the M, 21,500 standard, with an apparent M, of ~23,000. This protein was previously identified in MCP cartilage as IGF-BP-6 by Western immunoblotting and its preferential affinity for IGF-II over IGF-I (10). The identity of the bands was further verified by lowering the exposure time of the films to a third of the time shown in Fig. 1 (lower exposure = 24 h); the typical cartilage IGF-BP-6 doublet observed in previous studies (10) was evident in the cartilage samples. The smaller M, band had a similar migration velocity to the M, 21,500 standard and a lower intensity.
ED$_{50}$ was $\approx 10$ nM, indicating that IGF-I and IGF-II are equipotent in the competition with IGF-I tracer for binding to cartilage sites.

**Competition of Des-(1–3)-IGF-I with $^{125}$I-IGF-I for Binding to Cartilage Discs**—Des-(1–3)-IGF-I is a truncated, naturally occurring form of IGF-I that has been shown to have an average of only $\approx 1\%$ of the affinity of native IGF-I for the IGF-BPs, while retaining affinity of intact IGF-I for the signaling IGF-type I receptor (16–19). The des-(1–3)-IGF-I variant lacks the 3 amino-terminal amino acids, and systematic studies have shown that removal of Glu$^3$ accounts for the dramatic loss of binding affinity for the IGF-BPs. We postulated that if binding of IGF-I to cartilage occurs through IGF-BP sites, des-(1–3)-IGF-I would be unable to effectively compete with $^{125}$I-IGF-I for binding to this tissue. In a competition experiment, native hrIGF-I from R & D systems or from Gropep showed 80–82% inhibition of $^{125}$I-IGF-I binding to cartilage at 50–100 nM (Fig. 3A). On the other hand, hr-des-(1–3)-IGF-I was unable to compete with the radiolabeled ligand at any of the concentrations tested, which included the 50–400 nM range (Fig. 3B). This experiment strongly suggests that the IGF-BPs are involved in the specific binding of IGF-I to cartilage.

**Competition of IGF-I and IGF-II with $^{125}$I-IGF-I for Binding to Cartilage**—To further test the hypothesis that IGF-BPs constitute the major binding sites for IGFs in cartilage, we set up an additional competition experiment between IGF-II tracer and the two unlabeled IGFs. The experiment was based on the following considerations. It is well documented in the literature that IGF-BP-6 is distinctive in that it displays a much higher affinity for IGF-II than IGF-I in competition experiments carried out in solution when IGF-II is used as the tracer, but this differential affinity is not as marked when radiolabeled IGF-I is used. The measured potencies of IGF-II/IGF-I for competition with $^{125}$I-IGF-II range between 20 and 70, while the differences are only 2-fold when $^{125}$I-IGF-I is used as the tracer (Ref. 19; for review, see Ref. 20). Another potentially important cartilage IGF-BP, IGF-BP-2, also displays higher affinity for IGF-II than IGF-I in solution studies, even though the differences on the average are not as pronounced as for IGF-BP-6: the reported potency ratios of IGF-II/IGF-I for IGF-BP-2 competition with $^{125}$I-IGF-II vary between 20- and 40-fold (19, 20). We reasoned that because of its endogenous IGF-BP content (IGF-BP-2 and -6), articular cartilage would probably show a preferential affinity for IGF-II versus IGF-I when $^{125}$I-IGF-II is used as a tracer. When IGF-II was used to compete with this tracer for binding to cartilage, the ED$_{50}$ for inhibition was 8 nM (Fig. 4), compared with an ED$_{50}$ of 160 nM when IGF-I was used as the binding competitor (potency ratio for IGF-II/IGF-I: 20). Together with the results obtained with the des-(1–3)-IGF-I analog, these results strongly suggest that endogenous IGF-BPs in native cartilage constitute a major pool of binding sites for IGFs in articular cartilage.

**Transport of $^{125}$I-IGF-I through Cartilage Discs**—These experiments were designed to test the ability of IGF-I to diffuse through the full depth of the cartilage discs, and to assess the role of specific binding of the growth factor in this process. In these series of experiments, consistent with the results of Fig. 2A, a bolus of 100 ng of IGF-I effectively competed with radiolabeled IGF-I for binding to cartilage discs in equilibrium binding experiments. This concentration of IGF-I was used to compete for binding of $^{125}$I-IGF-I in transport experiments. Fig. 5 (inset) shows a diagram of the transport apparatus. The $^{125}$I-IGF-I was added at the start of the experiment to the “upstream” compartment, allowed to diffuse through the 400-$\mu$m-thick cartilage discs, and the effluent radioactivity monitored in the “downstream” compartment. A total of nine transport
experiments were carried out with very reproducible trends. Fig. 5 shows a representative experiment in which the efflux of 125I-IGF-I into the downstream bath (as a percentage to the total added to the upstream compartment) is plotted as a function of time. The time lag \( t_{\text{lag}} \) before effluent counts were observed after addition of 125I-IGF-I to the upstream compartment was calculated to be 266 min, using the intercept on the time axis for the line fit to the first steady state (linear) portion of the data (400–620 min). In general, the time lag reflects the combined diffusion-reaction transport process governing emergence of a solute into the downstream bath (21). In this case, the lag would include the time that it takes for endogenous IGF sites to be saturated with the radiolabeled growth factor, after which a steady state rate of 125I-IGF-I diffusion is seen between 400 and 620 min. The diffusivity for IGF-I calculated from this steady state flux was \( 4.1 \times 10^{-7} \) cm\(^2\)/s (calculation of this diffusivity took into account the small amount of residual low molecular weight iodinated species in the 125I-IGF-I preparation, by means of separate control experiments as described in detail by Garcia et al. (14)). The diffusivity was used to estimate the \( t_{\text{lag}} \) that would be expected in the absence of binding (21). This theoretical \( t_{\text{lag}} \) is 11 min, 24-fold faster than experimentally observed, strongly suggesting that transport of IGF-I through these cartilage discs was dramatically slowed by binding of the growth factor to sites within the tissue. To further test this possibility, a bolus of unlabeled IGF-I was then introduced into both the upstream and downstream compartments after the steady state rate of efflux had been achieved, at \( t = 620 \) min. The concentration of unlabeled IGF-I, \( 1 \times 10^{-7} \) M (100 nM), was at least 100-fold higher than that of labeled material initially present in the upstream compartment. This resulted in a nearly 2-fold rate of increase in the efflux of 125I-IGF-I at \( t = 620 \) min (extrapolated from the initially linear portion of the curve), followed by a return to the previous steady state flux by 1400 min. This latter observation reflects the exchange of bound 125I-IGF-I for unlabeled IGF-I during the 195 min of accelerated linear efflux of radiolabeled material into the downstream compartment, followed by progressive saturation of these sites by unlabeled IGF-I. Addition of a second bolus of IGF-I at 1450 min did not further affect the rate of efflux, suggesting previous saturation of binding sites by the unlabeled growth factor added at \( t = 620 \) min. The binding properties exhibited by the articular cartilage discs during the non-equilibrium transport of IGF-I, particularly the competition for tissue sites by unlabeled IGF-I, are consistent with the role of the IGF-binding proteins delineated in the equilibrium binding studies.

**DISCUSSION**

Previous studies have shown that IGFs bind to various types of cells and their extracellular matrices produced in culture through IGF-BPs. In this study, instead of examining IGF...
binding to tissue structures produced in vitro, we examined the specific binding of IGFs to articular cartilage discs, and their transport across their depth. These tissues have an architecture largely assembled in vivo. The advantage of this approach is that it ensures that the tissue characteristics, including its porosity, three-dimensional molecular structures and interactions, local ionic character, etc., closely resemble the physiological situation. We present a line of evidence in support of the specific binding of IGFs to bovine articular cartilage through the endogenous IGF-binding proteins. First, the equilibrium binding experiments clearly showed dose-dependent, saturable competition of excess unlabeled IGFs with radiolabeled IGF for binding to cartilage discs. Second, the IGF-I analog, des-(1–3)-IGF-I, which has a very low affinity for IGF-BPs (an average of 1% of normal) but normal affinity for the IGF type I signaling receptor (16–19), is not able to compete with 125I-IGF-I for binding to cartilage. Second, the IGF-I analog, des-(1–3)-IGF-I, which has a very low affinity for IGF-BPs (an average of 1% of normal) but normal affinity for the IGF type I signaling receptor (16–19), is not able to compete with 125I-IGF-I for binding to cartilage. Third, when 125I-IGF-II is used as the binding ligand for articular cartilage, unlabeled IGF-II displays greater effectiveness as a competitor for displacement of binding than IGF-I (potency of IGF-II/IGF-I: 20). This differential affinity is not observed when 125I-IGF-I is used as the binding tracer. These observations are consistent with the expected solution binding activity of IGF-BP-2, and particularly IGF-BP-6 to the IGFs (20). Since these are major IGF-BPs in bovine cartilage (Ref. 10, Fig. 1), the findings provide evidence that binding occurs through tissue IGF-BPs.

It is important to note that, while the local tissue environment could affect the binding parameters in many ways, the endogenous IGF-BPs (presumably mostly IGF-BP-6) retain their preferential affinity for IGF-II; this points to an important role for this isoform in the tissue. A proposed model is that IGF-BP-6 has two binding sites for IGFs. One has weak affinity for the two IGFs, while the other has a much greater affinity for IGF-II than I (19). In binding studies, at low levels of IGF-II tracer, this high affinity site may be preferentially filled and preclude binding to the other site. The only other protein in cartilage known to have preferential affinity for IGF-II over IGF-I is the IGF-II/M-6-P receptor. The affinity of this receptor for IGF-I is 100–500-fold or less that for IGF-II (4, 22). In our studies, the affinity of IGF-I was only 20-fold lower than that of IGF-II (based on ED50 values for competition of the ligands with 125I-IGF-II), which is in the range of reported affinities of IGF-BP-6 for IGFII/IGF-I (potency ratio of 20–70) using this tracer (20). A slight contribution of the IGF-II receptor to the experimental observations cannot be strictly ruled out.

The results presented in this paper differ significantly from a previous proposal that IGFs bind to cartilage unspecifically through proteoglycans (23). In agreement with our study, this previous work showed a linear uptake of radiolabeled IGFs by
cartilage, but the authors did not use a specific competitor, and provided calculations based on the assumption that only signaling receptors would be responsible for specific binding. The present study demonstrates that most of the binding of IGF to the tissue is specific and strongly suggests the involvement of endogenous IGF-BPs in this process. The results are not inconsistent with the possibility that the IGF-BPs may in turn be bound to proteoglycans in cartilage. Heparan sulfate proteoglycans have been implicated in IGF-BP binding to extracellular matrices (24–26). IGF-BP-6 contains a peptide sequence (YRKRQCRS) within its thyroglobulin repeat region that conforms to the consensus sequence XBBBXBBX (X = non-basic amino acid, B = basic) for heparan sulfate binding in a number of proteins, and the IGF-BP-6 sequence has been directly shown to bind to heparin (24). IGF-BP-2 has a motif that also displays high heparin binding activity (BBBXX) in many proteins, and it has been shown that this IGF-BP binds to heparin in the presence of IGF-I, suggesting that a cryptic heparin binding site may be exposed following binding of the IGF-BP to IGF (25). Thus, a candidate anchor for the IGF-BPs is perlecan, a heparan sulfate proteoglycan present in the pericellular matrix of articular cartilage (27). It is also of note that IGF-BP-2 has been found to bind aggrecans through the chondroitin sulfate chains (28), but the specificity of this interaction remains to be defined. The localization of the IGF-BPs in articular cartilage will be the subject of a future study.

It is worth pointing out that the present binding studies were carried out under free-swelling, non-loading conditions. It is possible that either dynamic or static compression may alter the affinities of the tissue for the IGFs by altering the tissue structures to which they are bound, by water extrusion, fluid flow, fluid-generated electrical currents, matrix compaction, compression, and diffusion.

![Figure 4](http://www.jbc.org/)

**FIG. 4.** Competition of unlabeled IGF-I and IGF-II with 125I-IGF-II for binding to bovine articular cartilage. This experiment was similar to that shown in a bar format in Fig. 2, except that IGF-II is used as the binding tracer. For comparison, the data of Fig. 2 are replotted in a linear form as an inset labeled 125I-IGF-I. As indicated in the figure, for both the inset and the main figure, the symbols connected by solid lines represent the competition by IGF-I, and the symbols connected by dotted lines represent the competition by IGF-II. The upper horizontal dashed line across the graph indicates the binding of tracer in the absence of competitor, and the lower horizontal dashed line denotes the level at which saturation is reached by the IGF-II competitor (the unspecific binding component); these lines were used as boundaries to denote the specific binding component and evaluate the ED50 values for competition by the unlabeled IGFs.

![Figure 5](http://www.jbc.org/)

**FIG. 5.** Diffusion-reaction kinetics and steady state diffusion flux of 125I-IGF-I across cartilage. Inset, transport chamber. The cartilage discs are sealed using gaskets and O-rings to assure that all solute and fluid transport occurs across the tissue between the two compartments. Fluid in both compartments is magnetically stirred and recirculated with return ports close to the tissue in order to further minimize stagnant layer effects on transport (for further details, please refer to "Experimental Procedures" and Ref. 14). Main panel, normalized downstream concentration of 125I-IGF-I is plotted as a function of time. A bolus of radiolabeled IGF-I was introduced into the upstream compartment at time, t = 0 (indicated by the left-hand side arrow). The time at which measurable amounts of 125I-IGF-I were seen in the downstream compartment is given by Tlag = 266 min. At t = 620 min, unlabeled IGF-I was added to both compartments up to 10^{-7} M (middle arrow), resulting in an exchange of the unlabeled IGF-I with radiolabeled IGF-I reversibly bound to the tissue, followed by an eventual return to the previous steady state flux (evident between 1200 and 1400 min). At t = 1450 min, another bolus of unlabeled IGF-I was added to both compartments (right-hand arrow), and no change in flux was detected. C*, concentration in downstream bath; C*, concentration in upstream bath.
that the subsequent linear, steady state rate of efflux (by equilibrium with the concentration of radiolabeled ligand, and available sites in the tissue gradually attain reversible binding the time lag for 125I-IGF-I efflux into the downstream bath, tissue. A likely explanation for this observations is that during tissue that initially slow the transport of IGF through the calculations (21), pointed to the existence of binding sites in the of radiolabeled IGF-I transport can by enhanced nearly 2-fold by the continued diffusion of radiolabeled IGF through the tissue. This likely represents the release of bound 125I-IGF-I from tissue sites by exchange with the unlabeled ligand entering the tissue from both sides, combined with the continued diffusion of radiolabeled IGF through the tissue.

The experimental findings from these transport experiments, together with the binding competition data of Figs. 2–4 suggest that specific binding of IGFs to cartilage (mostly through the IGF-BPs) regulate the paracrine activities of the IGFs in articular cartilage.

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