IGF-I and IGFBP-3 transport in the rat heart

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Submitted 26 July 2002; accepted in final form 15 August 2002

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THE HIGHEST CONCENTRATION of IGFs is in the blood, where the IGFs are present mainly as a 150-kDa ternary complex composed of IGF, IGF-binding protein (IGFBP)-3, and an acid-labile subunit. This complex prolongs the half-life of IGF-I and IGF-II in the blood, presumably because the 150-kDa complex does not easily cross the endothelial boundary to sites of IGF action. The endocrine role of the circulating IGFs and the mechanism(s) whereby IGFs traverse the endothelium have not been defined clearly (4, 6).

In the present report, we have used the isolated, beating rat heart to study the transendothelial transport of the perfused binary IGF-IGFBP-3 complex. The isolated rat heart was chosen because its perfused surface is ~90% microvessels, the capillaries have tight interendothelial junctions, the preparation requires small perfused volumes, and the capillary endothelium has distinct and specific binding sites for IGF-I.

We demonstrate the presence of IGFBP-3 binding sites in the perfused heart and examine the roles of IGFBP-3 and the IGF-I binding sites in the transport of perfused IGF-I to cardiac muscle.

MATERIALS AND METHODS

Cell binding. Microvascular endothelial cells were prepared from bovine heart adipose tissue, as previously described (9). In vitro binding studies were performed with confluent microvascular cells on 12-well Linbro trays (ICN, Aurora, OH). 125I-labeled IGF-I or 125I-IGFBP [2 × 104 counts/min (cpm)] was added to 12-well trays alone or with unlabeled IGFs or IGFBPs. After 90 min at 22°C, the monolayer was removed with 0.1 N NaOH and counted in a gamma counter. Data are expressed as means ± SE in three wells. Each experiment was performed three times.

Preparation of chimeric IGFBP-43 and IGFBP-34. The basic COOH-terminal 20 amino acids of IGFBP-3 (P3 region) and the homologous domain of IGFBP-4 (P4 region) were “swapped out” to generate the IGFBP-43 and IGFBP-34 chimeras, as previously described (9). The genes encoding the chimeric binding proteins were excised from the cloning vector and ligated into the EcoRI/XhoI sites of the vector pBacPAK9 (Clontech, Palo Alto, CA) for the expression and production of the chimeric IGFBPs in a baculovirus system. IGFBP-43 and IGFBP-34 were purified from media of infected High-Five insect cells.

Heart perfusion. Adult male Sprague-Dawley rats, weighing ~300 g (Harlan Sprague Dawley, Indianapolis, IN), were anesthetized, the chest cavities opened, and the hearts removed and suspended by a perfusion catheter placed in the aorta. Perfusions were done for 1 min at a flow rate of 3 ml/min. The hearts were perfusion fixed, embedded in paraffin, and prepared for autoradiography as previously described (2, 9). After 6–10 wk, the sections were developed, and the number of silver grains overlying subluminal cardiac muscle was assessed. For consistency, counts were done in

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the outer region of the midportion of the left ventricle, near large arteries where perfusion is optimal. Data are expressed as the number of grains per unit area. All animal studies were approved by the Veterans Affairs and University of Iowa Animal Care Committees.

RESULTS

We used several agents to identify the presence of specific binding sites for IGFBP-3 in the perfused heart. $^{125}$I-IGFBP-3 and -IGFBP-43, both of which have been shown to bind specifically to cultured microvessel endothelial cells (5, 9), were perfused through the hearts. IGFBP-43 is a chimera of IGFBP-4 in which a 20-amino acid basic COOH-terminal portion of IGFBP-4 has been “swapped” with the homologous region of IGFBP-3. IGFBP-4 3, like IGFBP-3, binds to cultured microvessel endothelial cells with high affinity. $^{125}$I-IGFBP-3 and -IGFBP-4 were tested in the perfused heart, because neither binds well to cultured microvessel endothelial cells (5, 9), whereas both bind to IGF-I with high affinity. $^{125}$I-IGFBP-34 and -IGFBP-4 were tested in the perfused heart, because neither binds well to cultured microvessel endothelial cells (5, 9), whereas both bind to IGF-I with high affinity. Each $^{125}$I-IGFBP (3, 43, 4, and 3) was perfused through beating hearts ($n = 3$) and counted for $^{125}$I grains (Fig. 1). As in cultured cells, hearts perfused with IGFBP-3 and IGFBP-43 retained a substantial number of $^{125}$I grains, whereas hearts perfused with IGFBP-4 and IGFBP-34 retained <15% of that contained in hearts perfused with IGFBP-3 or -4 (Fig. 1).

These findings suggest that the perfused heart, as well as cultured microvessel endothelial cells, has specific binding sites for both IGF-I and for IGFBP-3. We next examined the potential roles of each binding site in the transendothelial transport of perfused IGF-I and the perfused binary complex of IGF-I and IGFBP-3. It has been previously shown that both perfused IGF-I and IGFBP-3 cross the endothelial boundary and localize predominantly in cardiac muscle (1). Hearts were perfused with $^{125}$I-IGF-I alone, $^{125}$I-IGF-I plus IGFBP-3, and $^{125}$I-IGF-I plus the IGF-I analog Long R3 (LR3) IGF-I. LR3 IGF-I binds to type 1 receptors for IGF-I but binds poorly to IGFBPs. When $^{125}$I-IGF-I alone was perfused in the beating heart, $^{125}$I grains in cardiac muscle averaged 608 grains/unit area (Fig. 2). When $^{125}$I-IGF-I was perfused with LR3 to block binding to the IGF receptors, $^{125}$I grains in muscle decreased to 58. $^{125}$I-IGF-I perfused with IGFBP-3 in a molar ratio of 1:10 (IGF-I to IGFBP-3) retained 510 grain counts, not statistically different from $^{125}$I-IGF-I alone (608). When $^{125}$I-IGF-I and IGFBP-3 were infused with LR3 to block IGF-I receptors, grain counts decreased but remained substantially higher than the perfusion of $^{125}$I-IGF-I and LR3 IGF-I (252 vs. 58). This suggested that IGFBP-3 could “chaperone” IGF-I, possibly through the IGFBP-3 binding sites. When $^{125}$I-IGF-I was perfused with IGFBP-34, $^{125}$I grains were 131 or 26% of IGF-I perfused with IGFBP-3. Finally, when LR3 was added to the perfusion of $^{125}$I-IGF-I with IGFBP-34 to block the type 1 IGF receptor, grain counts in muscle were reduced to their lowest level at 24/unit area.

The binding of the binary complex of $^{125}$I-IGF-I/IGFBP-3 to cultured microvessel endothelial cells was tested to determine whether the $^{125}$I-IGF-I-IGFBP-3 complex can bind to IGFBP-3 binding sites as an intact binary complex. $^{125}$I-IGF-I (30,000 cpm/well) was exposed to microvessel endothelial cells by itself, with unlabeled IGF-I (1 µg/ml), and with IGFBP-3 at a 1:10 molar ratio of $^{125}$I-IGF-I to IGFBP-3. Maximal binding occurred with $^{125}$I-IGF-I alone at 7%. With excess unlabeled IGF-I, binding was decreased to 0.4%. When $^{125}$I-IGF-I was present at a 1:10 ratio of $^{125}$I-IGF-I to IGFBP-3, the same ratio as in the perfused heart, binding of $^{125}$I-IGF-I was decreased to 0.8%.

DISCUSSION

The isolated beating rat heart contains specific microvessel binding sites for IGF-I and responds to IGF by modulating glucose metabolism and lactic acid production. We demonstrate that the beating rat heart
also has specific binding for IGFBP-3 and that these binding sites may contribute to the transport of IGF-I to cardiac muscle, the presumed target of IGF-I bioactivity in the heart. The presence of IGF binding sites was demonstrated by perfusion of hearts with IGFBP-3, -4, -4, and -3. IGFBP-4 binds to cultured endothelial cells with equal to or higher affinity than IGFBP-3. It acquires this property even though most of its structure is identical to IGFBP-4, which does not bind to endothelial cells. In contrast, IGFBP-3 in structure, binds weakly to cultured endothelial cells. When perfused through the heart, IGFBP-3 and IGFBP-4 were retained in the hearts, whereas IGFBP-4 and IGFBP-3 were minimally retained.

The concept of the IGF-I binding sites and IGFBP-3 sites playing a role in the transport of perfused IGF-I to underlying cardiac muscle was suggested in several ways. For IGF-I receptors, when 125I-IGF-I was first perfused by itself, substantial IGF-I was transported to cardiac muscle. The same study was repeated with the addition of LR3 IGF-I, an IGF-I analog known to block IGF-I receptors. This decreased the appearance of IGF-I in cardiac muscle to ~10% of that with perfusion of 125I-IGF-I alone. We next examined the potential role of the IGFBP-3 binding sites in the transport of perfused IGF-I. When 125I-IGF-I was perfused as the binary complex IGF-I-IGFBP-3 in a molar ratio of IGF-I to IGFBP-3 of 1:10, transport was decreased to 41% of IGF-I alone, which was not statistically different from perfusion with IGF-I alone. When IGF-I binding sites were blocked by the addition of LR3 IGF-I, transport of IGF-I in the presence of IGFBP-3 was decreased to 41% of IGF-I alone, which was significantly more than perfusion with IGF-I plus similar concentrations of LR3 IGF-I (41 vs. 10%). This suggested that the IGFBP-3 can chaperone IGF-I through the IGFBP-3 binding sites. However, it should be noted that, in cultured endothelial cells, 125I-IGF-I-IGFBP-3 complexes at a 1:10 ratio, a similar ratio as in the perfused hearts, bound minimally to the IGF-I receptors, suggesting that binary complexes in the beating heart may behave differently than when exposed to cultured cells.1

Free IGF-I and IGF-I-IGFBP-3 binary complexes are present in the circulation (10), and the binary complex has been used in therapy (7). However, >90% of all IGF-I in the blood is contained in the ternary complex of IGF-I-IGFBP-3/acid-labile subunit (4). If free IGF-I and the binary complex of IGF-I-IGFBP-3 are the only forms of IGF-I that do cross the endothelial boundary, then results of the present study are obviously relevant. However, if IGF-I in the ternary complex does have an endocrine function, then the mechanisms whereby IGF-I or IGF-I-IGFBP-3 is freed from the 150-kDa complex need to be defined to better understand the mechanisms and control of such processes in understanding the normal and pathological physiology of IGF.

This work was supported by funds from Veterans Affairs research and by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-25421 and DK-25285.

REFERENCES