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IGF RECEPTORS IN MYOCARDIAL CAPILLARY ENDOTHELIUM: POTENTIAL REGULATION OF IGF-I TRANSPORT TO CARDIAC MUSCLE

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Beating rat hearts were perfused with ^{125}I-IGF-II alone or ^{125}I-IGF-II and unlabeled IGF-II or insulin, then prepared for radioautography. Maximal ^{125}I-IGF-II grain counts over capillaries were decreased in a dose-dependent manner by unlabeled IGF-II but were unaffected by coperfusion with insulin. To determine a potential role for capillary receptors in the transfer of circulating IGF to cardiac muscle, the effects of sequential loss of capillary IGF binding sites was determined. For IGF-I, loss of capillary binding sites by trypsin perfusion was accompanied by proportional decreases in the subsequent appearance of IGF-I in cardiac muscle. In contrast, similar decrements of capillary IGF-II binding did not affect muscle levels of IGF-II. We conclude that capillary endothelium of the intact heart possesses distinct IGF-I and IGF-II binding sites, with the capillary IGF-I binding sites being of potential importance in the transfer of vascular IGF-I to subendothelial cardiac muscle.

Cultured endothelial cells and isolated microvessels possess distinct surface binding sites for the closely related peptide hormones insulin, IGF-I and IGF-II (1-3). In addition, microvessel endothelium of intact tissues, such as the capillaries of perfused hearts, also contain insulin and IGF-I receptors with similar affinities and specificities as in cultured endothelial cells (4,5). In the present study, we present evidence that the capillary endothelium of the perfused rat heart also possesses surface binding properties characteristic of the type II IGF receptor. To determine the potential role of the heart capillary IGF receptors in the transfer of IGF from the vascular compartment to the subendothelial cardiac muscle, capillary IGF binding sites were destroyed by trypsin perfusion of the heart, and the subsequent appearance of IGF in cardiac muscle was determined. Increasing loss of endothelial binding sites for IGF-I was accompanied by proportional decreases in the appearance of IGF-I in cardiac muscle. In contrast, similar decrements of capillary IGF-II binding was not associated with significant change in cardiac muscle concentrations of IGF-II. Taken together, these studies suggest that capillary endothelial receptors for IGF-I, but perhaps not IGF-II, may play a role in the transfer of IGF to cardiac muscle.

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METHODS AND MATERIALS

1. Iodination of IGF-I and IGF-II: IGF-I was obtained from Amgen Biologicals (Thousand Oaks, CA) and purified IGF-II was a generous gift of Dr. Rene Humbel (Zurich, Switzerland). IGF-I and IGF-II were iodinated by the method of Van Obberghen-Schilling *et al.* (6) and purified as previoulsy described (7). Specific activities of the preparations used in this study ranged from about 200-300 *u*Ci/*u*g protein.

2. Heart perfusion was performed on isolated, beating rat hearts using previously decribed procedures (5). Hearts were perfused with ^{125}I-IGF, alone or with unlabeled IGFs, for a period of one minute. The one-minute perfusion period was utilized for all experiments for two major reasons: 1) it represented the earliest period in which accurate, reproducible grain counts could be assessed without excessivley long storage periods for grain development (i.e.<10 weeks) and, most importantly, 2) with perfusion times exceeding one minute, there was progressive degradation of ^{125}I-IGF-II in cardiac muscle (data not shown), which would obviate or diminish relevance of grain counting in the muscle compartment. a. ^{125}I-IGF: Grain counting over muscle cells and capillaries was carried out using an ocular grid and two cell compartments were analyzed: 1) Muscle cells: Grains were counted over randomly selected areas consisting entirely of myocytes within the ocular grid boundaries (25 u^2). This cellular compartent comprised > 90% of cardiac tissue. 2) Capillaries: Randomly selected circular profiles of capillaries (average volume density 7.5% of total heart tissue) were analyzed by counting grains within ~ $\pm$ 0.3u (one grain diameter) diameter of the capillary endothelial boundary. The half-distance (HD) of grain distribution has been estimated at ~ 0.2 u. Therefore the vast majority of grains associated with capillaries are included in this compartment. Since the average heart capillary under these conditions measured 4.8 u in diameter (range 3.2-9.0 u of > 150 vessels from each of 3 separate hearts), the average area analyzed per capillary was approximately 4.5 u^2. In each case, the density of background silver grains was subtracted from the density of silver grains over cells to give the specific number of grains per unit area. For each datum point, 100-150 individual determinations were made from each of three separate hearts and reported as the average $\pm$ SEM. Density of grain counts over capillaries was 5-15 fold greater than over muscle per unit area in all studies. b. Trypsin treatment: Hearts were perfused with several concentrations of trypsin (Difco Laboratories, Detroit, MI) ranging from 0.05% to 0.70% for 1 minute, followed by perfusion with buffer for 30 seconds. The hearts were then perfused with [^{125}I]IGF (~ 1 ng/ml) for 1 minute and processed for radioautography as previously described. Several sections of myocardium were prepared from each trypsin-treated heart for assessment of morphologic integrity by electron microscopic evaluation. Perfusion with trypsin did not alter the heart beat rate.

3. Cultured microvessel endothelial cells: Endothelial cells from bovine periaortic adipose were prepared by modifications of the technique of Kern *et al.* (8). The endothelial nature of the cultures was documented by characteristic morphology and uptake of the endothelial cell marker dil-Ac-LDL. ^{125}I-IGF-I binding studies were performed with adherent cells in 12 well trays as previously described (1).

RESULTS

The binding of ^{125}I-IGF-II to heart capillary endothelium is illustrated in Figure 1, left panel. Maximal binding of ^{125}I-IGF-II was progressively decreased when the labeled tracer (~ 1 ng/ml) was coperfused with increasing amounts of unlabeled IGF-II. IGF-II at 10 ng/ml decreased ^{125}I-IGF-II binding to ~62% of maximum and at 500 ng/ml to 21% of maximum. In contrast, unlabeled insulin, coperfused at concentrations up to 100,000 ng/ml, had no significant effect on ^{125}I-IGF-II binding to the heart capillary endothelium. At the electron microscopic level, the vast majority of ^{125}I grains within the capillary compartment were directly associated with or within 1/2 grain diameter of endothelial cells.

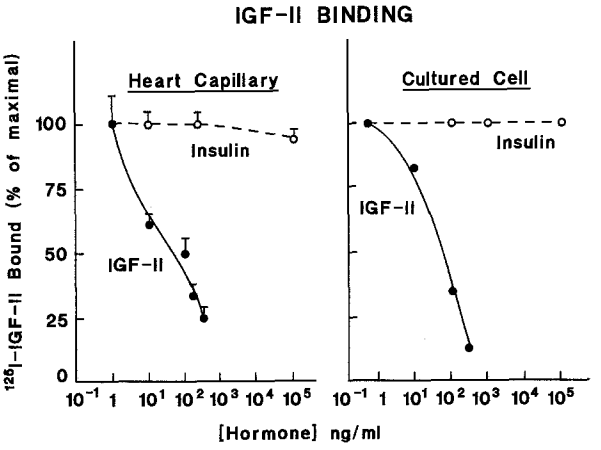

Figure 1: Binding of [^{125}I]-IGF-II to heart capillaries of perfused rat hearts (left) and to cultured microvessel endothelial cells (right). For heart studies, [^{125}I]IGF-II (1 ng/ml) was perfused alone or with varying concentrations of unlabeled IGF-II or insulin for 1 min and autoradiographic grain contents determined as described in Methods and Materials. Each point represents the mean ± SEM of data from 3 separate hearts. For cultured cells, binding studies were performed in adherent cell monolayers (1).

The right panel of Figure 1 illustrates IGF-II binding to cultured microvessel endothelial cells. IGF-II binding to the cultured cells yielded similar affinities of unlabeled IGF-II and insulin for the ^{125}I-IGF-II binding sites, despite the differences in experimental binding conditions used for the heart perfusion and cultured cell studies (1 minute perfusion at 37° for the heart studies and 1 hour incubation at 22° for the cultured cells).

A potential role for the capillary binding sites for IGF-I and IGF-II in the transfer of IGFs from the intravascular space to cardiac muscle was next evaluated in 2 ways: 1) assessment of both capillary endothelial and cardiac muscle ^{125}I-IGF concentrations (Figure 2) and 2) determining the effect of trypsin destruction of capillary binding sites on the subsequent appearance of IGF in cardiac muscle (Figure 3). First, ^{125}I-IGF-I or ^{125}I-IGF-II was perfused alone, with increasing concentrations of unlabeled homologous hormone or with increasing concentrations of insulin. After 1 minute perfusion, the hearts were fixed and subsequently analyzed for ^{125}I grain distribution within the capillary and muscle compartments (Figure 2). The binding of ^{125}I-IGF-I and ^{125}I-IGF-II to capillary endothelium (Figure 2, left panel), as previously published for IGF-I (4) and previously shown for IGF-II in Figure 1, is characteristic of Type I and Type II IGF receptors. The patterns of IGF-I and IGF-II binding in cardiac muscle are similar to that observed in the capillaries.

We next attempted to gain some insight into the transfer of IGF to the cardiac muscle by partially destroying capillary binding sites with trypsin then reassessing the appearance of perfused ^{125}I-labeled IGF in cardiac muscle (Figure 3). For IGF-I, preperfusion with increasing concentrations of trypsin up to .5% trypsin resulted in proportional decreases in ^{125}I-IGF-I in capillary and muscle compartments. At trypsin

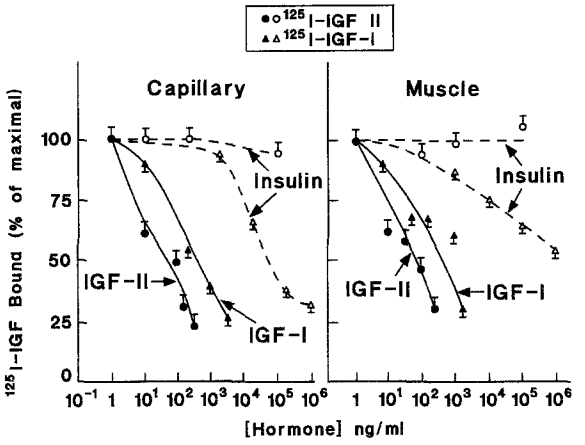

Figure 2: IGF binding over capillaries (left) and cardiac muscle (right) in hearts perfused with [^{125}I]-IGF-I (Δ , ▲) [^{125}I]-IGF II (O , ●) alone or [^{125}I]-IGF with increasing concentrations of unlabeled IGF or insulin. Data represent the mean ± SEM for 3 separate hearts with 100-150 grains counted per datum point per heart. IGF-I data in the left panel have been previously published (4).

concentrations > .5%, capillary binding of IGF-I continued to decrease whereas muscle ^{125}I-IGF-I dramatically increased. Thus, grain densities for IGF-I and II in muscle, which were initially 10-20% of capillary grain density, approach or exceed capillary grain density at trypsin perfusion of 0.7% (e.g. initial muscle/capillary grain density for IGF-I studies in Figure 3 was 1.25/6.3 per 25 u^2, while after 0.7% trypsin the ratio was 2.75/1.6. We have also previously demonstrated that preperfusion of trypsin at concentrations > 0.5% was associated with morphologic changes of the capillary endothelium and leakage of proteins with molecular sizes in the range of the IGFs, such as insulin (9). The IGF-II binding data post trypsin perfusion (Figure 3, right panel) differed from the IGF-I data.

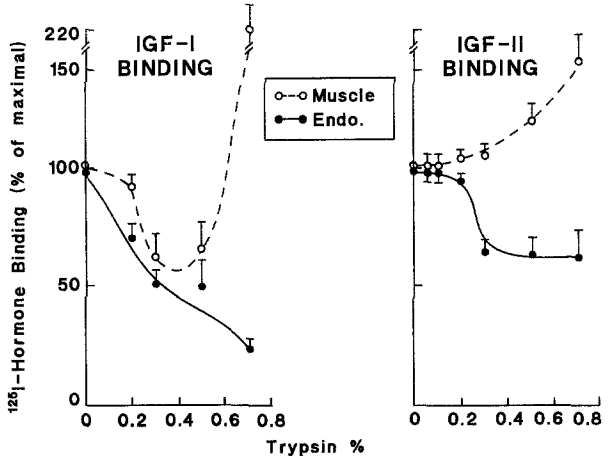

Figure 3: Effect of trypsin preperfusion on subsequent IGF-I binding (left) and IGF-II binding (right) to capillary endothelium (●——●) and cardiac muscle (O--O).

With decrements of 30-40% in capillary binding of IGF-II at 0.3 or 0.5% trypsin, there was no change in cardiac muscle levels of ^{125}I-IGF-II.

Finally the nature of the ^{125}I grains has been simultaneously assessed under all experimental conditions. Portions of hearts or entire hearts were rapidly frozen and sonicated in the presence of protease inhibitors (10nM N-ethyl maleimide, 0.92 mM phenylmethylsulfonyl fluoride, 1 mM bacitracin, 0.1% Triton X-100). The solubilized radioactivity, which was $\geq$ 70% of total heart radioactivity, was analyzed for degradation of the labeled peptide by precipitability in 5% trichloroacetic acid (TCA) and chromatographic pattern on Sephadex G-50 columns. In all cases, $\geq$ 90% of ^{125}I-IGF (I and II) was intact, hormone.

DISCUSSION

In this study, we demonstrate the presence of IGF-II binding sites on capillary endothelium of the perfused rat heart. The IGF-II binding sites had high affinity for IGF-II and no crossreactivity with insulin, properties similar to IGF-II receptors previously characterized in cultured microvessel endothelial cells and isolated microvessels (1-3). Thus, the capillary endothelium of the intact, beating rat heart has distinct binding sites for insulin (5), IGF-I (4) and IGF-II. Increasing evidence indicates that the heart capillary endothelium plays a central role in the transport of intravascular insulin to cardiac muscle, a known target tissue for both insulin and the IGFs (9). In these studies (9), the role of the endothelial receptors in insulin transport to cardiac muscle was indicated by the effect of partial destruction of binding sites by trypsin and, more importantly, by selective blockade of capillary binding by anti-receptor antibodies that were not significantly transferred to the cardiac muscle compartment. In the present study, we determined the effect of trypsin destruction of capillary binding sites on the subsequent appearance of ^{125}I-IGF in cardiac muscle. Trypsin treatment resulted in differing effects for IGF-I and IGF-II. While loss of 30-50% of capillary IGF-I binding caused proportional decreases in muscle IGF-I, 30-35% loss of capillary IGF-II binding sites did not affect muscle levels of IGF-II. This finding implies that at least a portion of the intravascularly-derived muscle IGF-I required initial capillary endothelial binding in order to reach the cardiac muscle, whereas capillary binding of IGF-II may be less relevant to the transfer of IGF-II from the vascular space to cardiac muscle. However, we feel that the IGF-II data obtained in the trypsin perfusions must be interpreted with caution. In these studies, capillary binding sites for IGF-I appeared more susceptible to destruction by trypsin than IGF-II binding sites, independent of the concentration of trypsin (Figure 3). Such differential vulnerability of IGF capillary binding sites could affect muscle levels of the IGFs and account, in part, for the unaltered muscle levels of IGF-II in the trypsin studies. Therefore, the potential relevance of the capillary binding sites for IGF-II in IGF-II transfer to cardiac muscle, we believe, remains uncertain. Finally, it must also be emphasized that the data obtained with trypsin treatment in the present study cannot be extrapolated to in vivo conditions for several reasons including

the nonspecific effects of trypsin and the absence of IGF-serum binding proteins in the perfusing solutions.

It is now apparent that the interactions of capillary endothelium with the IGFs are multifaceted and likely to be quite complex. Cultured capillary endothelial cells possess characteristic IGF-I and IGF-II receptors as well as atypical insulin-IGF receptors (10), serve as target tissues for a diverse group of IGF effects (11-13) and are capable of producing highly specific, low molecular weight IGF-binding proteins (14). The findings of the present study begin to characterize a preparation in which the complex consequences of IGF-endothelial interactions can be potentially dissected in an intact, functional system.

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