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Disposition of Radiolabelled Insulin-Like Growth Factor I (IGF-I), IGF-II and Their N-Terminal Truncated Variants in Rats

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THE MAJORITY of circulating insulin-like growth factors I (IGF-Is) and IGF-IIs are bound to a family of high affinity binding proteins, IGF binding proteins (IGFBPs). These IGFBPs are considered to modify the bioavailability of IGFs, and can inhibit and enhance IGF-I action under various conditions *in vitro* [1]. The complex of IGF-IGFBPs has a relatively long half-life and is present in the intravascular space [2, 3]. N-terminal truncated variants of IGF-I and IGF-II, des (1–3) IGF-I [4, 5] and des (1–6) IGF-II [6], are biologically more potent than intact IGF-I and IGF-II, respectively, because these variants have reduced affinity for the IGFBPs but their affinity for the type-I IGF receptor is similar to that of intact IGF. Des (1–3) IGF-I has been identified in a variety of tissue extracts and biological fluids [7–10], whereas des (1–6) IGF-II has not yet been found. Our recent demonstration of a protease in the rat serum which is capable of generating des (1–3) IGF-I from IGF-I [11] suggests that N-terminal truncation of IGFs regulates the bioavailability of these growth factors. In the present study, we compared the serum half-life, tissue uptake and urinary excretion of these truncated IGF variants with those of their intact precursors.

Materials and Methods

^{125}I-labelled IGF-I, des (1–3) IGF-I, IGF-II and des (1–6) IGF-II (2×10^6 cpm each) was bolusly injected into the left jugular vein of male Sprague-Dawley rats (BW 250–300 g) under ketamine anesthesia. 200 μl blood samples were obtained from the right femoral artery at 1, 3, 5, 10, 15, 30, 60, 120, 180 and 240 min after the injection. Urine samples were collected at 30 min intervals via the implanted bladder catheter. Tissues were prepared on ice immediately after exsanguination by decapitation at 2 h after the injection. The radioactivity of these samples was measured with a gamma counter.

Results

The serum disappearance curves of the radiolabeled peptides are shown in Fig. 1. Mean (± SEM) values of the half-life of des (1–3) IGF-I, IGF-I, des (1–6) IGF-II and IGF-II were 20.5 ± 4.0, 228.3 ± 22.0, 21.3 ± 1.1 and 181.7 ± 13.3 min, respectively.

The tissue uptake of the radiolabeled peptides is shown in Fig. 2A. Maximal accumulation of all 4 radiolabeled peptides was found in the kidneys. The tissue uptake for ^{125}I-IGF-I was distributed in the following order: the kidneys > pancreas > small intestine > liver > duodenum > stomach > the lungs > spleen > heart > large intestine > testes > brain > skeletal muscle. A similar pattern was found with ^{125}I-IGF-II. The tissue uptake ratio of truncated IGFs to intact IGFs is shown in Fig. 2B. Skeletal muscle, kidneys and testes showed a preferential uptake of both truncated variants. Intestines, duodenum and stomach showed preferential uptake of des (1–6) IGF-II. Both the pancreas and brain favoured uptake of intact IGF-I compared to des (1–3) IGF-I. The ratio of des (1–6) IGF-II to

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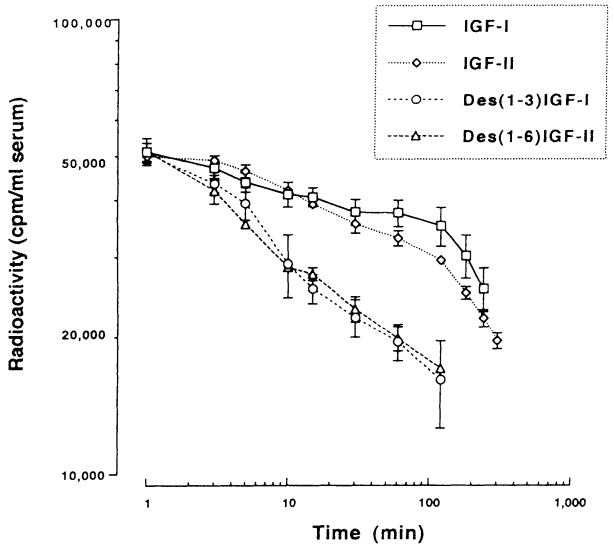

Fig. 1. Disappearance of ^{125}I-labeled IGF-I, des (1–3) IGF-I, IGF-II or des (1–6) IGF-II from the circulation in the rat. Residual radioactivities in serum were measured after a bolus i.v. injection of either radiolabelled peptide (2×10^6 cpm). Mean ± SEM values for three rats are shown.

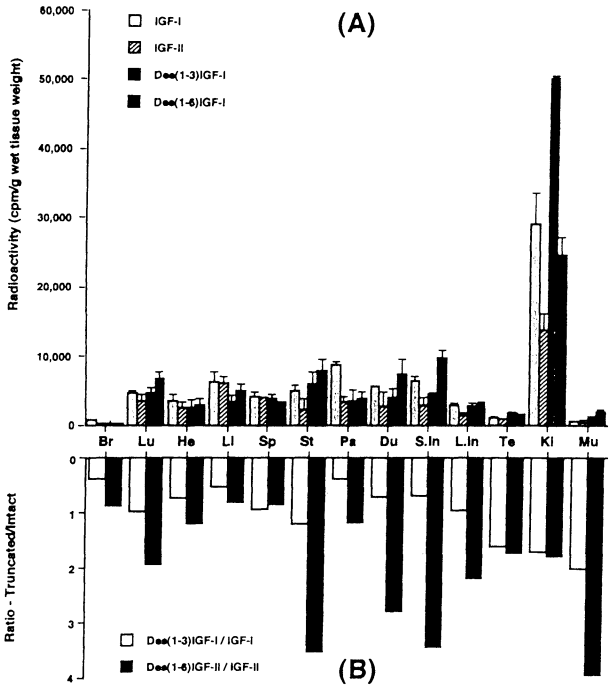

Fig. 2. A) Accumulated radioactivity in rat tissues at 2 h after a bolus injection of radiolabeled peptides (upper pannel). B) The tissue uptake ratio of truncated IGF to intact IGF (lower pannel). The abbreviations are: Br, brain; Lu, lung; He, heart; Li, liver; Sp, spleen; St, stomach; Pa, pancreas; Du, duodenum; S.In, small intestine; L.In, large intestine; Te, testis; Ki, kidney; Mu, skeletal muscle.

intact IGF-II was lower in the spleen, liver and brain.

The accumulated radioactivity in the urine was measured at 30 min interval. Urinary excretions of radiolabelled truncated IGF-I and IGF-II for 120 min were much greater than those of intact IGF-I and IGF-II, respectively (% excretion of injected peptide: 3.0 *vs.* 1.3%, 5.0 *vs.* 0.7%).

Discussion

The recent identification and characterization of an acid protease capable of generating N-terminal truncated IGF-I have renewed interest in the physiological relevance of this variant [11]. The presence of des (1–3) IGF-I in a variety of tissues and biological fluids [7–10], the demonstration of enhanced biological activity of this variant in both *in vitro* and *in vivo* assays [4, 5] and the observation suggesting that the proteolytic activity which generates this IGF-I variant is inversely regulated by growth hormone [12] suggest that the generation of des (1–3) IGF-I is unlikely to represent an unimportant degradation pathway. Furthermore, although des (1–3) IGF-I has not been detected in plasma, it is unlikely to be an artifact resulting from tissue disruption during extraction since it is also present in breast milk and possibly in urine [10, 13]. The earlier failure to demonstrate truncated variants in IGF preparations derived from plasma may be explained by the fact that the truncated variants of IGF-I and -II are rapidly cleared from plasma. The serum half-life of radiolabeled IGF-I, determined in the present study by means of a relatively simple protocol, is in close agreement with that reported in the literature. Ballard *et al.* [14] reported that a clearance rate for des (1–3) IGF-I was approximately 5 fold greater than IGF-I. The half-life of ^{125}I-des (1–6) IGF-II had not been previously determined. We found that the $t_{1/2}$ for radiolabeled IGF-II was similar to that of IGF-I, which is comparable with the previous report that the clearance rate for IGF-II is similar to that for IGF-I in adult rats: 1.34 ± 0.17 *vs.* 1.11 ± 0.13 ml/min per kg [14].

The kidney is the major organ for clearance of the IGFs and their variants from the circulation. Of the tissues examined, the brain, the pancreas and the liver showed more uptake of IGF-I than des (1–3) IGF-I. These data suggest that the IGFBPs are important for specifically facilitating tissue uptake in these tissue. In contrast, skeletal muscle and testes showed uptake des (1–3) IGF-I greater than IGF-I, indicating that the ability to bind to the IGFBPs inhibits uptake in these tissues. This tissue specificity may result in the relative abundance of the different IGFBPs expressed in the tissue or capillaries, or in the interaction of circulating IGFBPs with tissue capillary endothelium [15]. Differences in the tissue uptake and the subendothelial localization of IGF-I coupled with various IGFBPs have been demonstrated [16]. Tissue uptake would also be dependent upon the abundance of the IGF receptors. More marked differences were apparent when tissue uptake of des (1–6) IGF-II and IGF-II were compared. Since des (1–6) IGF-II has reduced affinity for the IGF type 2 receptor compared to IGF-II, as well as reduced affinity for the IGFBPs, the relative proportions of type 1 and type 2 receptors in different tissues may also be important in determining the tissue uptake and action of des (1–6) IGF-II.

The present findings demonstrate that the truncated variants of IGF-I and IGF-II are rapidly cleared from circulation in the rat primarily through the kidneys. The physiological significance of preferential uptake of the truncated variants by some tissues and the different uptake pattern for des (1–3) IGF-I and des (1–6) IGF-II remains to be further investigated.

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