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INCREASE IN THE TYPE 2 INSULIN-LIKE GROWTH FACTOR RECEPTORS IN THE RAT KIDNEY DURING COMPENSATORY GROWTH

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Summary: We have observed an increase in binding of IGF-I and IGF-II to microsomes obtained from rat kidneys undergoing compensatory growth following contralateral nephrectomy. This increase was evident by the 4th day and it preceded observable growth of the kidney. Binding returned to control levels just prior to the flattening of the growth curve of the kidney. The increase was due to an increase in the type 2 binding sites, the only type unequivocally present, from 95.3 ± 2.7 to 117.2 ± 4.1 pM/150 μg of microsome protein at its maximum at 4 days.

The insulin-like growth factors (IGFs) are peptide hormones, isolated from human plasma and believed to be necessary for mediating the growth promoting effect of growth hormone. They are produced by a wide variety of tissues (2,3) which may also be their targets of action. This supports the hypothesis that IGFs exercise all or at least part of their action through paracrine or autocrine mechanisms (4) and raises the interesting possibility that IGFs may be coordinators of growth at the local level, also involved in growth phenomena independent of growth hormone. The observation that the tissue content of somatomedin-C/IGF-I is substantially increased in rat kidneys undergoing compensatory growth following contralateral heminephrectomy (5) may be an example of this. In the adult animal, removal of one kidney is followed by a rapid increase in size of the remaining organ, consisting mostly of hypertrophy, but also involving some hyperplasia (6,7). The factor stimulating this growth is not known, but plasma growth hormone and somatomedin C levels remain unchanged (5).

We conducted the present study to examine the presence of possible modulation of the IGF receptors during this phenomenon.

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Materials and Methods

Animals: A left nephrectomy was performed on adult male Sprague-Dawley rats under pentobarbitone anesthesia, using a midline abdominal incision. Sham operations consisted of the same steps up to placing a ligature around the renal vascular stalk which was then removed without tying. The animals were fed at libidum and killed at the designated time intervals by decapitation. The right kidneys were removed, stripped of their capsule, and frozen at -20°C until further processing.

Membrane preparation and binding assay: Microsomal preparations were obtained by homogenizing the entire kidney in 0.3M sucrose, centrifuging the homogenate at 9,000 xg for 20 min, and then pelleting the supernatant at 100,000 xg for 90 min. The microsomal pellet was resuspended and washed once in 25 mM Tris buffer, containing 10 mM $MgCl_2$, and adjusted to pH 7.4. Further washing, even for prolonged periods, failed to increase binding, indicating no interference from endogenous ligands.

Binding assays were performed by incubating 150 µg of membrane protein with labelled ligands plus various amounts of unlabelled material, at 4°C for 18 hours, in a total volume of 0.3 ml. Binding observed in the presence of a large excess of ligand was considered non-specific and was subtracted from all other values. It was usually 10-20% of bound radioactivity.

Ligands: IGF-I and IGF-II were purified by high pressure liquid chromatography from outdated human plasma as previously outlined (8). They were iodinated with chloramine-T to a specific activity of 150-170 ci/gm. Purified porcine zinc insulin was iodinated by the same method. Tracers were purified either by gel filtration (insulin and IGF-I) or absorption to and elution from human placental membranes (IGF-II) (9).

Receptor Characterization: The molecular type of the receptor to which IGF-II bound was characterized by polyacrylamide gel electrophoresis, as described by Massague and Czech (10). Molecular radius, displacement by insulin, and possible presence of subunits were examined by photoaffinity crosslinking of the receptor to radiolabelled ligand, solubilizing, and running on a 5-15% gradient gel after reduction with dithiothreitol (DTT).

Statistics: Data are presented as means $\pm$ standard error of the mean. Differences between groups were assessed by the non-paired student "t" test.

Results

Binding studies: In a preliminary experiment, specific binding of radiolabelled IGFs and insulin to microsomal membranes was studied at day 5 post nephrectomy. The results are shown in Table 1. Compared to the sham-operated controls, IGF-II specific binding is significantly increased. IGF-I binding, although considerably lower, displays the same proportionate rise post contralateral nephrectomy. Insulin binding on the other hand, does not significantly change. Since IGF-II binding to these membranes appeared to be the predominant one, we proceeded to further characterize it and correlate it to the changes in kidney weight.

Table 1.
Binding of the three radiolabelled ligands to kidney microsomes from heminephrectomized animals (Nx) and sham operated controls

Ligand	Nx	Sham controls	p
IGF-I	5.1+0.2	3.2+0.2	<0.05
IGF-II	21.5+1.4	17.4+0.8	<0.05
Insulin	3.2+0.7	3.6+0.3	NS

The binding was measured on 150 µg of microsomal membranes (IGFs) or 250 µg (insulin) in a total volume of 0.3ml. Incubation was at 4°C, overnight.Binding in the presence of 500 ng of IGF-II is subtracted as non-specific.

Figure 1 shows the time course of the increase in kidney mass superimposed upon the changes in IGF-II specific binding. Compared to the sham operated controls, kidney weight in the heminephrectomized animals appears to increase by the fourth postoperative day, the first time point studied. This difference, however, does not become statistically significant until day 8. The increase continues until day 18, at which point it appears to reach a plateau, at a level that is approximately 50% higher than in the controls.

By contrast, a statistically significant difference is seen in the binding of IGF-II to microsomal membranes at the first time point tested, 4 days after the operation. This difference has declined by 8 days, and becomes non-significant thereafter. Therefore, an increase in IGF-II binding appears to precede compensatory kidney growth by a few days. It is also seen to decline just prior to the cessation of growth.

Scatchard analysis, performed on the 5-day IGF-II binding data, shows that the difference in binding can be accounted for by an increase in receptor

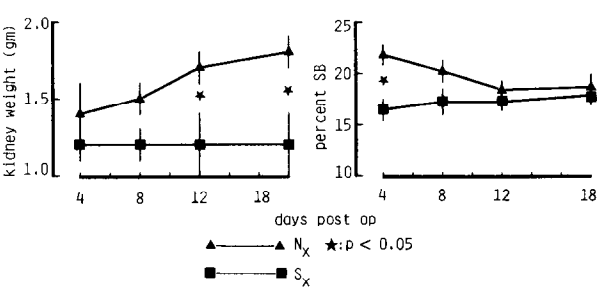

Figure 1.
Time course of the increase in kidney weight and IGF-II specific binding. Binding conditions as in table 1.

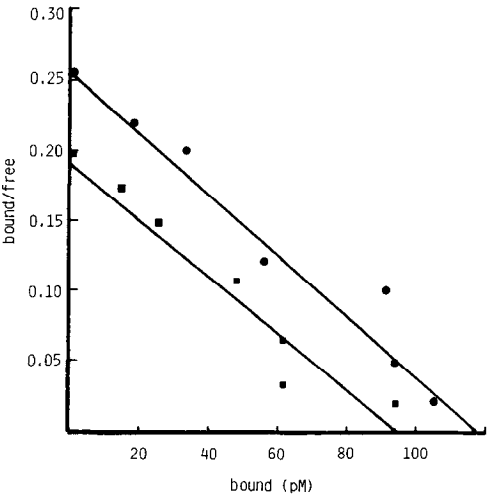

Figure 2.
Scatchard plots of binding of IGF-II to microsomal membranes (150 µg/0.3ml). Incubation conditions were as in figure 1.

number, the affinity remaining essentially the same (fig. 2). Mean concentration of binding sides was 117.2 ± 4.1 per 150 µg of protein in the nephrectomized animals, as compared to 95.3 ± 2.7 in the controls, while affinity was comparable at 2.1 ± 0.1 vs 2.1 ±0.2.

Characterization of receptor type: Unlabelled IGF-I was tenfold less potent in displacing IGF-II binding and insulin was non-reactive, suggesting a type 2 IGF receptor. This was confirmed by the results of the polyacrylamide gel electrophoresis, showing that the specific binding was confined to a molecular radius of 270 kilodaltons. In keeping with what is known about the type 2 IGF receptor, insulin was ineffective in competing for this site. The specific binding of radiolabelled IGF-I was much weaker. It was also confined to the 270 kilodalton band, without any evidence of a 95 kilodalton subunit in the reduced gels. This confirms that the binding that we studied was to a type 2 IGF receptor (Fig. 3).

Discussion

The mechanism of the compensatory increase in the mass of the remaining kidney following unilateral nephrectomy is poorly understood, despite extensive research on the subject (7). The initiating stimulus is believed to be humoral. Growth hormone and somatomedin concentrations in plasma do not change

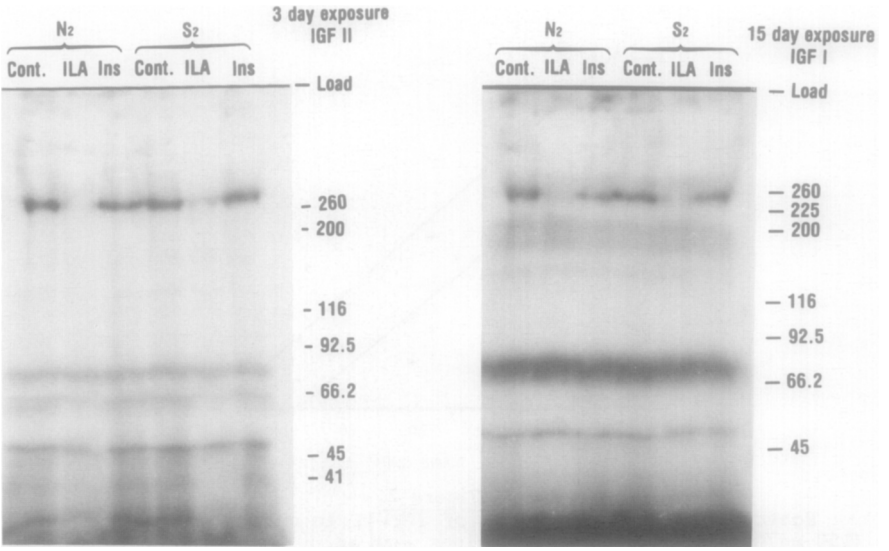

Figure 3.
Polyacrylamide gel (5-15% gradient) electrophoretic pattern of the kidney microsome receptor after crosslinking with each tracer, solubilization and reduction with dithiothreitol. The bands represent autoradiography of tracer on photographic film. Molecular radius standards were visualized with Coomassie blue staining. Insulin-like activity (ILA) refers to a semipurified preparation containing both IGFs.

(5), as one might expect from the fact that the growth stimulation is confined to the kidney. In the adult animal, the growth consists mostly of hypertrophy, as judged by protein and DNA content of the whole kidney (6). However, increased DNA synthesis is definitely present and an increase in mitotic activity mostly at the level of the proximal renal tubule is seen early in the course of the phenomenon (11).

We have shown that this compensatory increase in kidney mass is associated with an increase in the number of the type 2 IGF receptor binding sites in microsomal membranes obtained from the growing kidneys. Since IGFs have a mitogenic activity in a large variety of tissues, it is reasonable to expect that an increase in receptors for these petides would represent a growth stimulus. At this point, we do not know whether the increase we have observed is causally linked to the growth stimulation in the kidney, but the fact that the time course of the binding changes precedes observable growth, makes it unlikely that it is an artefact of the increase in the kidney mass or alterations in the proportions of cell populations with the kidney.

Stiles et al have reported that the tissue content of somatomedin C/IGF-I substantially increases early and transiently during compensatory kidney growth (5). We were unable to demonstrate type 1 IGF receptors in this organ in the rat, but IGF-I does bind to the type 2 receptor, albeit with a lower affinity. It appears, therefore, likely that at least some of the biologic effect of somatomedin C/IGF-I is mediated through binding to the type 2 receptor. The importance of this type of IGF receptors in mediating growth has been challenged on the basis of **in vitro** observations on the H35 hepatoma cell line (12), but these receptors appear to play an important role in other **in vitro** systems (13).

The relationship between the increases in peptide content and receptor numbers is not clear at this point. IGFs are not known to upregulate their own receptors. Somatomedin C/IGF-I in high concentrations actually downregulates the type 1 receptor, with no effect on the type 2 receptor in vitro (14). More studies are obviously needed to clarify the complex interrelationships between these phenomena and their importance in kidney growth.

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