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CHARACTERIZATION OF THE BINDING OF A RAT SOMATOMEDIN TO RECEPTORS IN HUMAN PLACENTAL CELL MEMBRANES

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SUMMARY: The somatomedins presumably initiate their growth promoting effects by first binding to specific cell surface receptors in responsive tissues. The specific and high affinity binding of [^{125}I]-rat somatomedin to human placental membranes was saturable and reversible with a dissociation constant of 4.5×10^{-9} M calculated from Scatchard analysis of competitive binding experiments. Competition for [^{125}I]-rat somatomedin binding to placental receptors by other somatomedins and growth factors suggest a close structural relationship between rat somatomedin and the human somatomedin, insulin-like growth factor I.

INTRODUCTION

The somatomedins are a family of low molecular weight peptides, purified from serum, which are thought to mediate the biological actions of growth hormone on cartilage and other extraskeletal tissues (1,2). Studies have shown that serum levels of somatomedin are growth hormone dependent, and that these peptides exert a wide spectrum of growth-promoting activities in responsive tissues and cell culture lines (2,3). The somatomedins can be subdivided into two classes: the first class are those purified from plasma or serum which include human somatomedins A (4) and C (2), human insulin-like growth factors I and II (5), and rat somatomedin. The second class includes multiplication stimulating activity, MSA, isolated from the conditioned media of a rat liver cell line (8,9). At present, amino acid sequence data is only available for

Abbreviations used: rSM, rat somatomedin; IGF, insulin-like growth factor; MSA, Multiplication stimulating activity; NGF, nerve growth factor; SDS, sodium dodecyl sulfate.

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insulin-like growth factors (IGF) I and II (10,11) thus preventing direct comparisons between the peptides. However, it has been speculated that the various somatomedins might be structural homologues based on cross reactivity in a number of cell surface receptor systems (2,9,12).

In an effort to clarify the relationship of rat somatomedin (rSM) to other purified growth factors, we have characterized the binding of rSM to placental cell membranes, a tissue known to be rich in somatomedin receptors (13). Using a highly purified preparation of [^{125}I]-rat somatomedin, we have tested the ability of other somatomedins and hormones to interact with rSM on a specific cell surface receptor. Although receptor cross reactivity is not definitive proof of structural homology, the binding data obtained point to a close resemblance of rat somatomedin and the human IGF-I.

MATERIALS AND METHODS

Rat somatomedin was purified from the serum of rats bearing growth hormone secreting tumors as previously described (6). This material was equipotent, 36 Units/mg, with homogeneous MSA (7) in stimulating [$^{35}SO_4$] uptake into the cartilage of hypophysectomized rats (14). The rat somatomedin was approximately 60% as potent on a weight basis as purified IGF, 35 mU/mg insulin-like activity (15), as judged in a radioreceptor assay specific for somatomedins (13). Unless otherwise noted, this rat somatomedin preparation was used in all binding studies.

Partially purified rat somatomedin was iodinated by a modification of the chloramine T procedure (16) to a specific activity of 40-80 μCi/μg and was separated from free iodide and high molecular weight protein aggregates on Sephadex G-50. This preparation was further purified by complexation with placental cell surface receptors in an analogous manner to that reported by Megyesi et al. (17) for nonsuppressible insulin-like activity and Hall et al. for somatomedin A (18). The [^{125}I]-rSM was bound to particulate placental membrane fractions under conditions detailed below and eluted from its receptor with 0.1 N acetic acid. This resulted in the depletion of 90% of the radioactivity from the initial preparation and gave a radiolabelled ligand which migrated on SDS gels (19) predominantly as a single entity with an apparent molecular weight of 8000 (Figure 1). Between 70-90% of this preparation could be displaced from placental membranes by an excess of either unlabelled rSM or IGF (Figure 2a). Receptor purified [^{125}I]-rSM was used within 2 days of preparation.

Conditions for the binding studies were as follows: particulate human placental cell membranes were prepared as previously described (13) and used in all binding experiments at a concentration of 400 μg membrane protein/ml. Binding of receptor purified [^{125}I]-rSM was performed

in a 0.1 N TRIS buffer, pH 7.4, containing 0.25 M NaCl and 1% BSA (w/w). A specified amount of unlabeled peptide was added followed by addition of membrane (total volume per tube, 0.5 ml). After a 16 hr incubation at 4°C, triplicate or duplicate samples were centrifuged, the pellets washed once with 0.1 N TRIS buffer, pH 7.4 (1% BSA), and centrifuged again with the radioactivity in the membrane pellets determined.

Homogeneous rat MSA was the generous gift of Dr. P. Nissley. Human insulin-like growth factors were kindly provided by Dr. R. Humbel. Due to the scarcity of IGF a partially purified preparation was used in displacement studies. This material had a potency of 35 mU/mg in the rat fat pad assay or approximately one-tenth the potency, on a weight basis, of either homogeneous IGF I or II (15). The material used for displacement was presumably a mixture of IGF I and II. Iodination of IGF I or II was performed with homogeneous preparations, both with reported potencies of approximately 350 mU/mg (15). Porcine insulin was a generous gift from Eli Lilly and Co. Rat prolactin and growth hormone were gifts from the National Pituitary Agency.

RESULTS AND DISCUSSION

The binding of receptor-purified [^{125}I]-rat somatomedin to placental cell membrane was saturable and reversible. Steady state binding occurred by 6 hr at 4°C and was reversible by a 100-fold dilution of the assay mixture. After 16 hrs at 4°C, approximately 8% of the total radioactivity added bound to the placental cell membranes of which between 70-90% was specifically bound (see Figure 2A). Kinetic studies at higher temperatures resulted in an unacceptable decrease in specific binding as was reported earlier for the binding of insulin to placental membranes (13). Specific binding was maximal between pH 7.4-7.8 and was not affected significantly by the addition of $CaCl_2$ or $MgSO_4$ although phosphate buffers did reduce specific binding of tracer by 20% (data not shown). Similar results were reported by Marshall et al. (13) for the binding of human [^{125}I]-somatomedin C to placental membranes.

By a number of criteria the receptor purified radioligand employed in these studies appears about 90% homogeneous. Gel electrophoresis (19) of the tracer (Figure 1) reveals a discrete band of radioactivity that migrates with a molecular weight of approximately 8000. More importantly, as high as 90% of this ligand could be displaced by an excess of either rSM or IGF. Radiolabelled rSM prior to receptor puri-

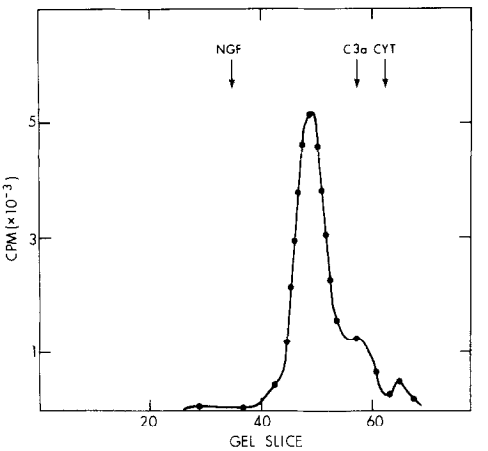

Fig. 1. SDS polyacrylamide disc gel electrophoresis (19) of receptor purified [^{125}I]-labelled rat somatomedin. Following electrophoresis the gels were sliced into 2 mm pieces and measured for radioactivity. The arrows indicate the position of proteins standards visualized in the gel following staining by Coomassie brilliant blue. Standards are as follows: CYT, cytochrome C, mol. weight 12,400; C3a, C3a-anaphylatoxi (23) mol. wt. 9300; and NGF, a synthetic fragment of NGF (24) mol. wt. ∿2300.

fication showed extremely low specific binding (less than 1%, data not shown).

In competitive binding experiments, an IGF preparation, 35 mU/mg (15) was essentially equipotent, on a weight basis, with rSM in displacing [^{125}I]-rat somatomedin from the placental receptor, as shown in Figure 2A. Within experimental error, the shape of the displacement curves were indistinguishable from one another over the concentration range studied. In a number of experiments the specific binding of [^{125}I]-rSM was 50% inhibited by between 2-3 μU of either IGF or rat somatomedin. This represents a concentration of approximately 20 ng/ml of IGF based on the reported activity of 300 mU/mg of homogeneous IGF-I or II as determined in the fat pad assay (15). Figure 2B shows a Scatchard analysis (20) of the competitive binding data presented in Figure 2A. A straight line is obtained from this plot, indicative of a single class of non-interacting receptor sites. On the basis of the

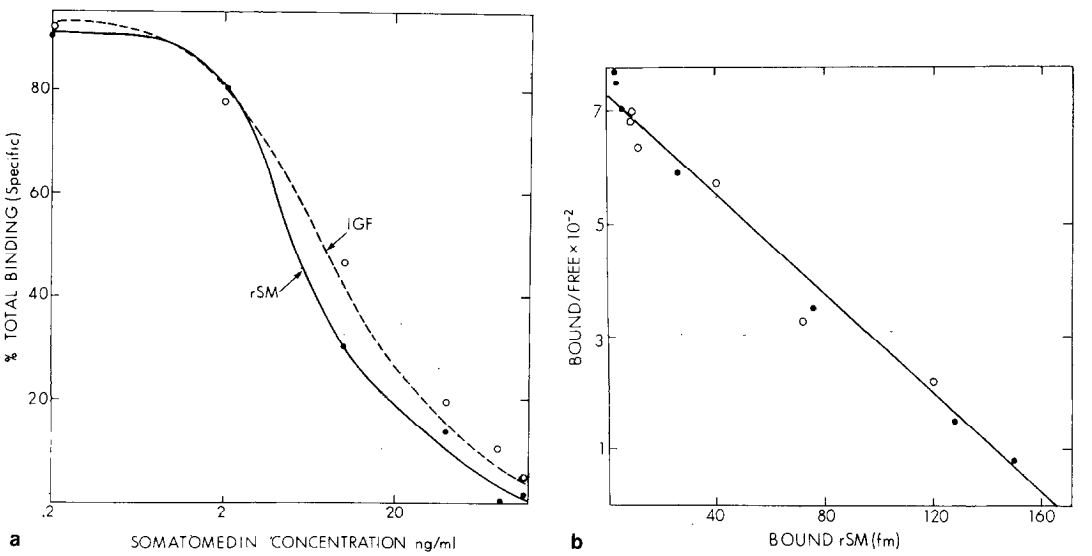

Fig. 2. A) Competitive binding studies with varying concentrations of rSM and IGF, 35 mU/mg (15), competing for the binding of labelled rSM to placental membrane. [^{125}I]-rSM, 56 fM/ml, was incubated with membrane (400 µg/ml) for 16 hr at 4°C to which increasing concentrations of unlabelled rSM (●—●) or IGF (O- -O) were added. For each point, radioactivity was determined for triplicate samples as described in "Materials and Methods." Nonspecific binding (20%) has been subtracted. B) Scatchard analysis of the binding data in (A). Both rSM (●) and IGF (O) concentrations have been plotted.

observed molecular weight of rSM of 8000, a binding constant of 4.5 x 10^{-9} M can be calculated from the slope of the Scatchard plot. In addition, if it is assumed that all the radioactive ligand is capable of binding to receptors, a binding capacity of 5.0 x 10^{11} rSM molecules/mg membrane protein is calculated from the abscissal intercepts of Figure 2B.

The specific interaction of other growth factors and hormones with the somatomedin receptor is shown in Figure 3. As seen, MSA, a somatomedin purified from the conditioned media of a rat liver cell culture line was less effective in displacing [^{125}I]-rSM from placental membranes than either IGF or rSM. In view of the fact that MSA and rSM exhibit significantly different isoelectric points (6,9), this binding data strongly suggests the presence of two distinct somatomedin peptides in the rat. That these rat peptides cross-react on the same placental

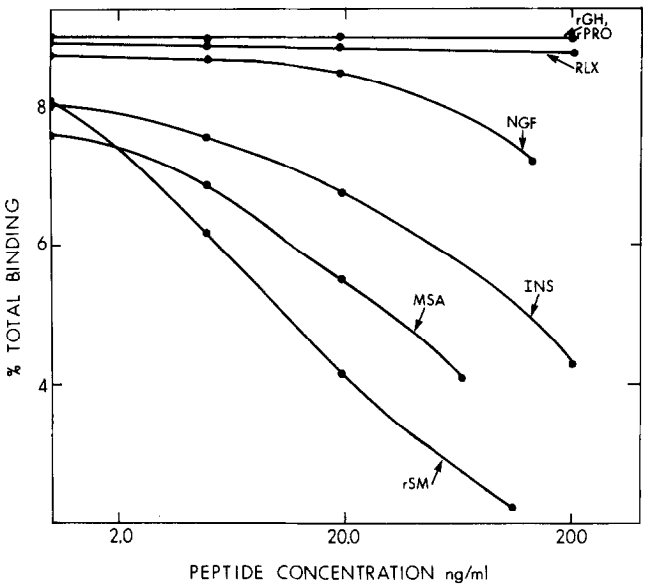

Fig. 3. Competition of various growth factors and hormones for the binding of [^{125}I]-labelled rSM to placental membranes. Tracer (62 fM/ml) was incubated with placental membrane (400 µg/ml) for 16 hr at 4°C with varying concentrations of the following peptides added at time zero: multiplication stimulating activity (MSA), rat somatomedin (rSM), insulin (INS), nerve growth factor (NGF), relaxin (RLX), rat growth hormone (rGH) and rat prolactin (rPRO). Radioactivity bound to the membranes was determined at the end of the incubation as described in "Materials and Methods." Nonspecific binding has not been subtracted.

receptor (Figure 3) suggests some structural similarity between the two molecules. As previously reported, MSA also competes with the human somatomedins, SM-A and IGF for receptors on liver (12) and chick embryo fibroblasts (9). Other peptides, as seen in Figure 3, compete weakly or not at all for placental receptor sites. Insulin, which bears structural homology to IGF-I and II (10), displaces [^{125}]-rSM at supraphysiological doses (500-1000 ng/ml). This finding was also observed in competitive binding studies using human somatomedin C as the radioligand (13). Nerve growth factor and relaxin, structural members of the insulin family of peptides (21,22), have marginal competitive effects at high concentrations, while rat prolactin and growth hormone cause no displacement of [^{125}I]-rSM.

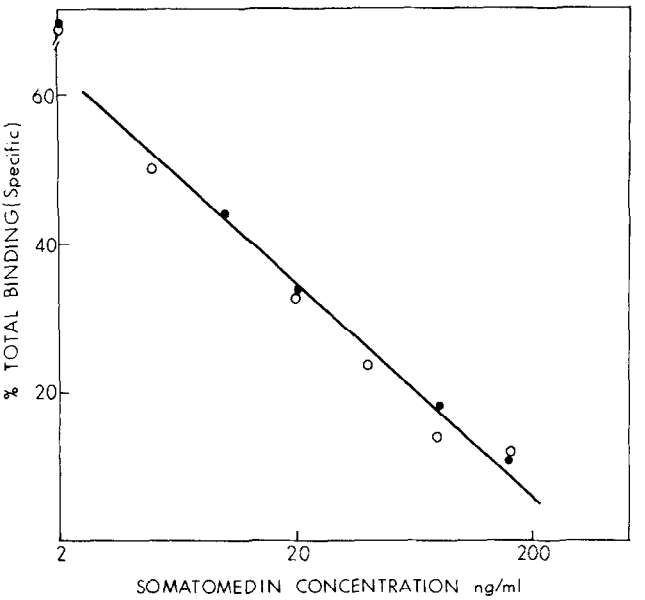

Fig. 4. Effect on binding of [^{125}I]-IGF-I to placental membranes by varying concentrations of unlabelled IGF, 35 mU/mg (0) and rSM (●). IGF-I was iodinated (15) to a specific activity of 20 μCi/μg, purified by Sephadex G-50 chromatography as described for labelled rSM in "Materials and Methods" and incubated with placental membranes (400 μg/ml) under identical conditions described for binding experiments using rSM as the radioligand. Nonspecific binding (10%) has been subtracted.

In addition to binding data discussed above several lines of evidence indicate a close structural resemblance between rSM and human IGF-I. In reciprocal binding experiments (Figure 4) rat somatomedin shows potent displacement of homogeneous [^{125}I]-IGF-I from the placental receptor. Additionally, procedures employed in the isolation of rat somatomedin (6) would select for an IGF-I-like peptide. These include the use of a radioreceptor assay employing [^{125}I]-IGF-I as the radioligand to monitor the purification of rat somatomedin, and the use of acromegalic rat serum as a starting source for the isolation of rSM. Recent evidence indicates that in human acromegalic states IGF-I accounts for up to 84% of the insulin-like activity in acid extracted serum (15). These data suggest that both human and rat serum contain a basic somatomedin peptide that are structurally related and conceivably, identical molecules.

MSA, a neutral peptide, is most likely a distinct somatomedin in rat serum.

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